Seed Testing INTERNATIONAL

ISTA News Bulletin No. 125 April 2003

Seed Testing in Hungary 48

The 27th ISTA Congress is to be held in the Eastern European Country. Dr. Ertsey gives us insight into their Seed Testing System

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Seed Testing
INTERNATIONAL

International Seed Testing Association (ISTA)

ISTA Secretariat
Zürichstrasse 50
P.O. Box 308
8303 Bassersdorf
CH - Switzerland

Phone: +41 1 838 6000
Fax: +41 1 838 6001
E-mail: ista.office@ista.ch
Internet: www.seedtest.org

Next Issue

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Other ISTA Publications

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www.seedtest.org

Editorial

By Michael Muschick,
ISTA Secretary General

Dear Reader,

What you now have in your hands is the ISTA News Bulletin with a new concept, a new cover and the new title *Seed Testing International*.

Seed Testing International should be a magazine by seed testers for seed testers. *Seed Testing International* will provide articles about and around seed testing.

Seed Testing International will mainly address the international challenges and developments but also describe regional challenges and developments and hence provide a colourful picture on all different regions and cultures of our ISTA family.

Seed Testing International will contain articles from our Executive Committee dealing with governance and political issues as well as from our Technical Committees dealing with improvements and developments in the area of seed science and technology. Moreover all our partner organisations around the world shall have a word in *Seed Testing International* describing challenges and developments from their point of view or in their region. I am proud to announce that in this first issue of *Seed Testing International* articles are published by ISF, APSA, ISHI and IPPC in order to present to you an interesting and vivid picture of the seed testing world and around it.

A focal point of this issue is the GM discussion. A hot topic which was already addressed in the last ISTA News Bulletin (December 2002 issue / No. 124). ISF provide their views on this very challenging issue, next to the detailed information on all our ISTA activities in that particular area, which are numerous. With these contributions we hope to provide you an overall picture of the different international and regional developments in this area.

Also plant health issues are an internationally challenging topic. Please read an update on the developments of the ISTA Plant Disease Committee as well as articles about ISHI and the IPPC (International Plant Protection Convention) on this subject.

I hope you are aware that the next ISTA Congress is coming up quite soon. The first announcement for this important event as well as an article about seed testing in Hungary can be found in this issue.

We sincerely hope that you will enjoy reading this first issue of *Seed Testing International* and are looking forward to receiving your comments/input on our new magazine as well as comments and questions on the different articles in this issue, which we would be pleased to publish in the next issue of *Seed Testing International*. Your feedback is important to us!

Before closing, I would also like to remind you that the next membership meeting is coming up very shortly: the ISTA Extraordinary Meeting 2003 to be held in Zurich, Switzerland from June 30 to July 3, 2003 and it will be my pleasure to welcome you personally in Switzerland at this important event.

Yours sincerely,
Michael Muschick

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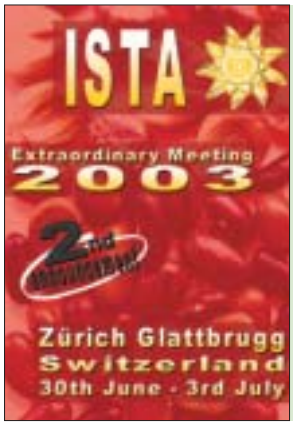

The 2nd Announcement of the Extraordinary Meeting 2003, to be held in Zurich, June 30 - July 3

Including Registration Form, and details about the meeting and Zurich

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Dr. Katalin Ertsey talks about Seed Testing in Hungary, where the 27th ISTA Congress is to be held in 2004

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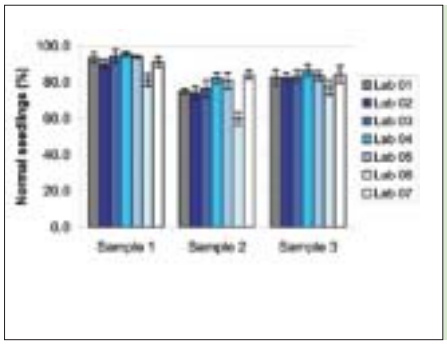

The ISTA Flower Seed Committee discuss the preparation of their ISTA Flower Seed Handbook, including Working Sheets of *Cyclamen* and *Petunia*

The ISTA GMO Task Force send out the samples for the 2nd ISTA GMO Proficiency Test

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Seed Trade Association in Kenya hold Accreditation Workshop in Nairobi

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President's Report

By Norbert Leist, ISTA President

Dear ISTA Members

We can rely on our Secretariat to be efficient at all times; they even reached me electronically here at the workshop "on varietal verification and GMO detection" at the ARC Roodeplaat Vegetable and Ornamental Plant Institute near Pretoria, South Africa, reminding me to write this outstanding letter.

Our workshop here is an example of the fruitful cooperation between FAO and ISTA, whereby we provide the technical expertise and the organization, and FAO, the financial maintenance, i.e. travel expenses for the participants and the lecturers. Following the successful concept that had already been tested in Argentina in 2001 for South America, the first part of this workshop deals with varietal verification and hybrid quality by electrophoresis of storage proteins in theory and practice. Thereby, the participants practiced the isoelectronical focusing with hybrid maize, barley and sunflower, and rice under the experienced/approved instruction of Rainer Knoblauch, whereas Dr. Enrico Noli, leader of the GMO Task Force working group, 'Exchange of Information', excellently presented and instructed the PCR analysis. The 18 participants from seven African countries were highly motivated to learn the techniques and it was intensively discussed. Often in workshops, there is an interesting mixture of participants from governmental, company and other private seed testing laboratories, from regulatory officials and universities. This leads to very interesting discussions regarding highly actual topics.

We were pleased that Dr. Michael Larinde from FAO participated in this workshop not only as an observer but also with a pipette in hand. In this he was able to gain a direct insight into the efficiency of this event, which not only results into a network of communication, but leads to new friendships. Dr. Bettina Kahlert, Head of Technical Committee Administration from the ISTA Secretariat, was the responsible person for all organization, and was most successful. We would like to thank Dr. Graham Thompson, the assistant director of Plant Protection and Biotechnology ARC, and his colleague, Gurling Bothma, for providing the excellent working and lecture rooms, and their practical support on location. With this workshop South Africa contributed to the further development of the African continent.

Dear ISTA Members, your ISTA Executive Committee will also meet in Africa March 10 to 13, 2003. We are invited by our ECOM Member Jeffrey Luhanga to hold our ECOM Meeting in Malawi for the approval of ISTA internal items as well as for the preparation of the 2nd Extraordinary Meeting, Zurich, June 30 to July 3, 2003. Please find below a few topics selected from the full agenda. (Two of the topics listed below raised at the Extraordinary Meeting in Bolivia, were very remarkable and have to be continued):

1. The ISTA position paper regarding stronger restructuring, and reorientation between governmental dependency and independency, as a free and autonomous association, with all consequences for voting rights, methodology acceptance and financing. In Bolivia, the delegates clearly supported innovation in careful, gradual changes, as well as carefully monitor the exciting structures. This needs accurate considerations and decisions.
2. The presentations of all Technical Committee Chairs, at the Extraordinary Meeting 2002, were so excellent, brief and precise all at the same time that the Executive Committee would like, in future, to keep this format of the meeting agenda for following Extraordinary Meetings.
3. One important issue to discuss is the future information and publication policy, including in particular the electronic media. Also the use of the ISTA Logo by our members needs a clear regulation.

As a matter of course, the ISTA Accreditation as well as the progress of our Technical Committee work will have their own time in the meeting.

You will see that this full programme, decorated with many single items, fills the week. On Friday, the ECOM will be invited to a meeting with the national and regional representatives to intensify the cooperation between this region and ISTA, in regards to our goals defined at the congress in Angers to support Africa, Asia and South Africa.

As Member, you might be interested to know that the 2nd ISTA Proficiency Test on GMO Testing has begun and that the first samples have been sent to participating laboratories. Remarkable is also the restructuring of our ISTA web site due to the intensive cooperation between Patricia Raubo from the Secretariat and Jim Sheppard from Canada.

Finally, we would like to congratulate the Institute of Plant Breeding, Seed Science and Population Genetics, from the University of Hohenheim on their 125th Birthday Celebration. ISTA is most appreciative of this Institute, which is currently under the direction of Prof. Dr. Michael Kruse, whom we all hold in esteem as an energetic leader and member of our committees.

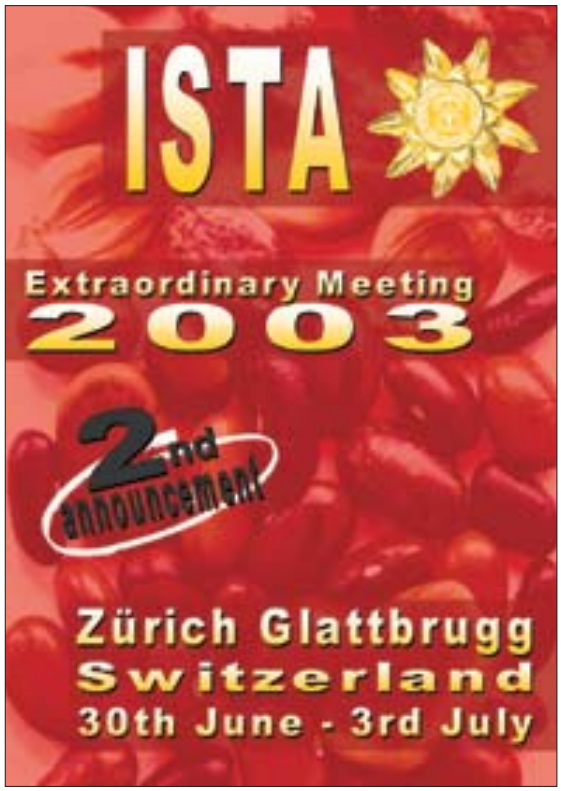
We think of Prof. Lakon who developed the Tetrazolium Test, and incorporated it into the ISTA Rules, also of Prof. Dr. Lindenbein, Dr. Bulat and Prof. Dr. Steiner, who took on important roles within ISTA, with great success. We have exciting role models in our Association, as well as many young, energetic Seed Testers, so that ISTA can reach it's goal successfully: Uniformity in Seed Testing.

I look forward to seeing all of you in Zurich at our 2nd Extraordinary Meeting, where I will be able to greet you personally.

Norbert Leist
President of ISTA

ISTA Extraordinary Meeting 2003

Zürich, Glattbrugg, Switzerland
June 30 - July 3, 2003



Dear Colleagues,

The International Seed Testing Association (ISTA) takes pleasure in inviting you to participate in its Extraordinary Meeting 2003, to be held from June 30 - July 3, in Glattbrugg, Zurich, Switzerland.

Following a decision of the ISTA Membership during the last Extraordinary Meeting in Santa Cruz, Bolivia, July 2002, the 2nd Extraordinary Meeting will be held in Switzerland. Through the Extraordinary Meetings, ISTA has been able to speed up its activities - something not possible from the previous three year meeting cycle - therefore we expect you will be joining us!

This meeting is aimed at discussing and deciding on proposals for changes to the ISTA International Rules for Seed Testing and business items of the Association, with the international participation of ISTA delegates and representatives from both the seed industry and governments, including experts in seed technology, scientific research and laboratory accreditation.

The main subjects of the meetings will be: Testing of GM Seeds - Chapter in the ISTA Rules and international harmonisation; Generic Method Validation Programme; ISTA Quality Assurance Programme; Accreditation of Laboratories world-wide - latest developments and international collaboration; Discussion on ISTA's relationship with governments.

THE OCCASION TO DISCUSS ALL YOUR TECHNICAL PROBLEMS AND CHALLENGES WITH THE BEST EXPERTS IN SEED TESTING FROM ALL PARTS OF THE WORLD.

Preliminary Programme

30 June - 1 July
Technical Committee Sessions

Sessions from all the ISTA Technical Committees, As well as longer sessions on Accreditation, Proficiency Testing, the Generic Method Validation Programme and the ISTA Rules.

2 - 3 July
Ordinary Meeting of the Association

Updates on invited partner organisations, consideration and adoption of activity reports of the Association's Committees, consideration and adoption of all changes to the International Rules for Seed Testing, consideration and adoption of current business matters of the Association, discussion on further activities and developments of the Association.

A full Preliminary Programme is currently available in the 2nd Announcement. However it is subject to change. The final programme will be published in the final announcement, once the Executive Committee has given its approval. For further information, or to receive the final announcement, please contact the ISTA Secretariat.

Travel Information

Regular scheduled flights from every continent and most countries and major cities of the world land at Zurich's international airport. The busy Hauptbahnhof, Zurich's main railway station, dates from 1871 and is located on Bahnhofplatz, in the city centre, and has excellent connections with both tram and bus lines. Trains run frequently from major Swiss centres. High speed Inter City Expresses also connect with most of the major cities within Europe, making travelling by train a viable option.

ISTA has determined to provide complimentary transportation from the airport to the hotel. The NOVOTEL Shuttle bus runs between the Airport and the hotel every 15 minutes. For participants arriving at Zurich's main railway station, there are direct trains from the station to the airport (the S7 and S2), where the shuttle can then be taken to the hotel.

Public Transport

A highly efficient and easy-to-use tram and bus network (known as Züri Linie) operates within the city, making sightseeing into Zurich city a pleasurable experience. Ferries also form part of Zurich's transport system. A boat trip is a must in summer. Short and extended cruises are available, and a sundowner on the Zurich Lake is the perfect way to spend a scenic afternoon.

Accompanying Persons

The accompanying person fee includes participation of the official dinner, lunches and coffee breaks. Please note that there is no official programme for accompanying persons, however guided tours and day trips can be arranged at short notice through the hotel. For further information, contact the reception desk at the Novotel, Zurich. ■

To register, please fill out the registration form enclosed herewith, and return to the ISTA Secretariat. You may also register online at

ww.seedtest.org

ISTA Extraordinary Meeting 2003 The Venue: Zurich, Switzerland

Zurich, Glattbrugg, Switzerland June 30 - July 3, 2003

In this part of the world, everything is that bit smaller, but with so many things to do, this simply means that you have all the more time for an unforgettable visit. Although the largest city in Switzerland, Zurich aptly promotes itself as the 'little big city' and has a historic centre compact enough to be explored on foot.

Zurich (or more familiarly, *Züri*) is situated in the heart of German speaking Switzerland, with a population of 363 000. Positioned at the northern tip of the Zürichsee (Lake Zurich), lakeside promenades and expensive houses are prominent and can be spotted along both shores. But the city's most familiar sites are, without a doubt, the Fraumünster and Grossmünster Minsters, which solemnly face each other across the River Limmat. The Old Town spans this river, and some of the most interesting lanes and buildings are clustered along its banks. In summer the view of the city is beautiful, with the lake reflecting the mountains and clear blue sky. The summer temperature averages at about 25°C and rarely rises above the 30°C mark. The humidity is at a comfortable level.

Zurich dates its origins from 15BC, when the Roman customs post of Turicum was founded. By the tenth century, the town had acquired the status of a city. The modern Zurich is a city of bankers in a country of banks. This concentration of wealth can most readily be seen along the Bahnhofstrasse, flanked by lime trees.

Other riches lie in the city's excellent universities - Zurich is a powerhouse for research, with public-private partnerships leading to innovations in design and the high-tech area. The city also has a strong cultural presence - over 30 museums, art galleries, auction houses, the opera, orchestras and the Schauspielhaus theatre.

The citizens enjoy a high standard of living, and this is evident in the many fashionable and enjoyable bars, cafés and restaurants that fill the Old Town. The ambience is heightened by the large swathes on either side of the River Limmat that are pedestrian-only areas. But for those who find the comfortable burgher lifestyle a little too tame, there are always alternative places to seek out. This is, after all, the city that saw the birth of the artistic movement of Dadaism - the antithesis of conformity.

The Extraordinary Meeting 2003 will take place at the NOVOTEL, Zurich Airport Messe, which is situated only 5 minutes from the Zurich International Airport in a quiet green belt area close to Zurich. The Novotel is only 12km from the city centre of Zurich, and easily reachable by public transport.

Key Attractions

Grossmünster
The twin towers of this attractive Minster - the largest in Zurich and the city's symbol - face onto the River Limmat and are best seen from Rathausbrücke.

Fraumünster
Although this beautiful Minster dates from the ninth century (when it was a Benedictine Abbey), it is often the five 20th-century stained-glass windows in the choir by Marc Chagall (1970) that attract visitors. These glass works of art are best seen in the morning light.

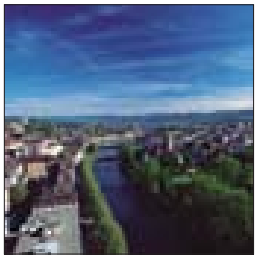
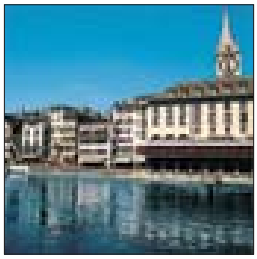
Kunsthaus Zürich (Zürich Art Gallery)
This is the city's most important art gallery, boasting a collection of paintings and sculptures by Swiss and international masters, covering most periods from medieval times but predominately from the 19th and 20th centuries. Two of Monet's Water Lilies paintings and the largest collection of Eduard Munch's works outside Norway can be found here.

Day trips/Excursions

Rapperswil
The beautiful little 'City of Roses' has a medieval character dating back to 1229, with romantic lanes and restaurants on the lakeside. It is located on the northern shore of Lake Zurich.

Jungfraujoch
From this mountainous location in the Bernese Oberland one can see Switzerland's largest glacier and the spectacular Alpine scenery of this region. The cogwheel mountain train from Lauterbrunnen climbs to the highest railway station in Europe at 3454m (11,333ft), on the Jungfraujoch. Bus trips can be arranged, or alternatively, a train trip will take about 5 hours, however, the Alps are always just outside the window.



27th ISTA Congress 2004
The Venue: Budapest, Hungary
May 13 - 24, 2004

Budapest, the capital of Hungary, is an economic, financial and cultural center with two million inhabitants. The city which is beautifully situated on both sides of the Danube river has a history dating back over 2000 years. There are ruins from the time of the Roman Empire as well as from the Middle Ages. Its main characteristics reflect the atmosphere of the end of the 19th century when the millennium of the Hungarian State was celebrated. It boasts a number of museums (picture gallery of the museums), theatres, concert halls, a lot of restaurants and other amenities. Several baths and thermal waters of various medicinal springs are also at the disposal of visitors. Budapest can easily be reached by air, train or car

Peaceful and bustling, a big metropolis and yet friendly, it treasures the old and embraces the new. Here the historic blends with the modern, the hills harmonize with the river, that is, the Danube, which flows through the city along a stretch of 28 kilometres.

Budapest has over 1,000 restaurants offering Hungarian and international cuisine. You will not have any difficulty in finding a place to eat which suits your taste and budget.

Development of the today face of Budapest between 1870 and 1900 - So fast was this growth that it earned the description of an 'American tempo'. However, Budapest resembled Chicago only in the speed of its growth. The development was carefully planned and the effect was delightful. In 1870 the municipality set up the Council of Public Works, which elaborated a grand master plan, and the city had the power to realize it. Everything that marked the standards of the age could be found in the master plan: there was a system of ring roads and boulevards, and a network of urban public transport: the height of the buildings was set, green spaces were included, and so forth. Though a major part of the city was built within the space of twenty years, the result was not monotony but a harmonious uniform style.

Up to the first third of the eighteenth century the left-bank settlement, the historic centre of the former town of Pest, consisted only of the district lying between today's Liberty Bridge, Chain Bridge, Múzeum körút and Károly körút, that is to say, it extended only as far as today's Kiskörút (Little Boulevard). The town was completely rebuilt and it grew gradually; today, of the original town of Pest, there only remain some parts of the fifteenth-century town walls, and from the eighteenth century, only the churches, as well as a few monasteries and public buildings. In contrast to the Castle District of Buda, which is by-passed by the main traffic of the city, the Inner City, with its shops, offices and important traffic arteries, is part of the city's everyday life.

The city is divided into two parts, the hilly side of Buda on the western bank and the flat plain of Pest on the eastern bank of the river Danube. These two parts of the city were once separate towns and were merged together with Ancient Buda (Óbuda) only in 1873. The name Buda Castle covers more than a castle or the Royal Palace in the capital city; it extends to the historical quarter full of sites. On bright spring days people invite friends for a "walk in the Castle", i.e. to wander around the Castle Hill quarter. The most exiting way of getting to the Castle is by taking the Funicular, a little cable car up the Castle Hill.

Places of Interest

Pál Valley Cave (Budapest)
This is a cave which was discovered in 1904 in the Rózsadomb area of Budapest. It is a hot spring cave and has been open to the public since 1919. Though the Pál Valley cave became noted for its exceptional stalagmites and stalactites, in reality the narrow, high, crevice-like passageways, the large differences in levels, and the sphere shaped forms resulting from hot water sedimentation are more characteristic of the place. The cave has 500 metres of built up pathway along which you see a variety of stalagmites and stalactites, the hot water sedimentary spheres and ancient shell shaped depressions. A pleasantly landscaped quarry area in front of the cave gives you a chance to rest after your labours in the cave.

Szemlohegy Cave (Budapest)
This cave was discovered in 1930 but only opened to the public in 1986. This cave is also the result of hot spring sedimentation and its most characteristic feature is the thick layer of mineral deposit covering the walls in shapes reminiscent of cauliflower and grapevine, which are known as peastones and are a wonderful sight. The special clean, dust free air in the cave makes it possible to operate cave therapy courses, thanks to which thousands of asthmatics and other sufferers of respiratory ailments find relief here.

Alcsút Arborétum (Alcsútdoboz)
This is a classical palace belonging to the Prince Palatine and designed by Mihály Pollack, though sadly only the palace façade and palace chapel remain.
The garden is an English landscape garden. There are several structures among the huge trees contemporary with the house, like beautiful bridges, the music pavilion, the child's house, the bear house, the water feature, and the Lourdes Cave. The reconstructed chapel has an exhibition entitled Historical Gardens of Hungary and in the child's house there is one on the Soldiers of the Archduke Joseph. Thanks to the work of the owners, the Palatine Joseph and the Archduke, the park today has a collection

ISTA Seed Symposium 2004

Budapest, Hungary, May 13 - 15, 2004

CALL FOR PAPERS

This is the first invitation to people interested in presenting a paper during the Seed Symposium of the 27th ISTA Congress under the theme

"Towards the future in seed production, evaluation and improvement"

Intending participants are encouraged to present papers and posters dealing with seed related topics. For the detailed scientific topics of the Sees Symposium, please refer to the information on page 6.

The following should be noted:

1. Papers should be of a high standard and should follow the usual format for scientific manuscripts with the subject matter arranged under 5 main headings:

- Introduction
- Materials and Methods
- Results
- Discussion
- Acknowledgements

(For exact details see the "Instructions to Authors" below.)

2. A summary of not more than one A4 page should be typed separately and should accompany the manuscript. The summary should give a clear indication of the topics covered and the main findings.

3. Please indicate the session (see topics of the Seed Symposium) in which the paper shall be presented.

4. Papers will be presented orally or in poster form, both of which have equal status. As the number of oral presentations will be limited by time constraints, oral presentation of your paper may not be possible, and you may be asked to present your paper as a poster. This decision will be made by the symposium organisers, and you will be advised accordingly by the end of January 2004.

5. Authors who wish to present a paper in poster format only (i.e. not be considered for oral submission) are not required to submit a full text of the paper. Instead they should submit a summary only, but indicate clearly in a covering letter that the paper is for poster presentation only.

6. Summaries of symposium papers will be given to all participants at the Congress. Complete manuscripts will be required by session leaders.

Deadline for Submission:
September 30, 2003

Papers must be sent to:

ISTA Secretariat
Seed Symposium 2004
Zürichstrasse 50
8303 Bassersdorf
CH-Switzerland

Tel: +41 1 838 60 00
Fax: +41 1 838 60 01

E-Mail
SeedSymposium@ista.ch

Instructions to Authors

For the ISTA Seed Syposium, 2004

1. Papers will be selected according to their originality, either of the methodology or of the results. They should be written concisely and with clarity.

2. Authors are reminded that acceptance of a paper at the symposium does not mean automatic submission to the journal 'Seed Science and Technology'. Authors who wish to submit a symposium paper to 'Seed Science and Technology' should follow the regulations for that journal and submit their papers independently to the editor.

3. For consideration for the symposium, authors should submit their papers in the following format:

(a) Papers should be written in English.

(b) The summary should be headed by the paper's title and the author's name(s) and address(es) and the number of the session to be presented in. It should be written in English and include information on the author's aim and major findings. It should not contain discussion or references and must not exceed 250 words. All summaries will be published prior to the symposium. Authors are kindly asked to submit the summaries as attachment via e-mail or on computer disk either CD or floppy disk

$3^{1/2}$. Suitable formats are as follows:

- Operating system: Windows X
- Word processing systems:
  Word, WordPerfect and ASCII;

(c) The paper (excluding the summary) should include the title, name(s) and address(es) of the author(s), the number of the session, the text, tables and figures. The text should contain the following:

Introduction should state the main reasons for undertaking the work, together with a clear definition of its purpose.

Methods used should be described with enough detail to show how the results were obtained (i.e. experimental design, treatments, operating conditions, materials used, methods of statistical interpretation).

Results should include statistical information (i.e. significance of results, coefficient of variation etc.).

Discussion should review the results in relation to the existing literature, to indicate their scientific or practical significance, and to come to a brief conclusion.

Tables and figures; results may be presented either in tables or figures, but in no case should information be presented in both ways.

The list of References must contain only the papers cited in the text, arranged in alphabetical order of the authors.

4. Authors are reminded that the purpose of submitting their paper is to allow the symposium organisers to decide:

(a) Whether the subject material and quality of the paper is acceptable for inclusion in the ISTA Seed Symposium.

(b) What subject area the paper should be assigned to.

(c) Whether the paper should be presented orally or in poster form.

The decisions of the organisers are not related to publication of the paper, but only to its symposium presentation. Therefore, authors may choose to either submit their paper in a format which meets all of the 'Seed Science and Technology' requirements, or submit the paper in a format which simply meets the requirements listed in 3(c). If the former option is chosen, authors are reminded that they must still submit their paper to the editor of 'Seed Science and Technology' for consideration for publication.

5. To give the symposium organisers time to decide on the programme and form of presentation, and the ISTA Secretariat time to preprint and distribute all the summaries (oral plus poster) and to prepare a symposium programme (giving paper titles, authors and time of presentation), authors are requested to meet the following deadlines:

(a) The text plus a separate summary page must reach the ISTA Secretariat by September. Papers received after this date will not be considered for inclusion in the symposium.

(b) Authors will be notified of the acceptance of their paper, the subject area to which it has been assigned, and whether it is to be presented in oral or poster form, by January 31, 2004

(c) Authors invited to present their papers orally must send the text of the spoken version of the paper (i.e. what will be said in the 10 minutes available) to their session chairperson and to the local organisers by February 28, 2004. Note that this is the text which will be used by the translators.

Host institute

National Institute for Agricultural Quality Control

Local Organisers

Dr. Károly Neszmélyi
President of the Organising Committee

Dr. Katalin Ertsey
Secretary of the Organising Committee
E-mail: ertseyk@ommi.hu

Symposium Convenor

Dr. Alison Powell
E-mail: a.a.powell@abdn.ac.uk

Scientific Correspondence

ISTA Secretariat - Seed Symposium
Zürichstrasse 50, CH-8303 Bassersdorf
Switzerland
Tel: +41 1 838 60 00
Fax: + 41 1 838 60 01
E-mail: SeedSymposium@ista.ch

Congress Secretariat

Diamond Congress Ltd.
Fõ u.68., H-1027 Budapest, Hungary
Tel: +36-1-214-7701, +36 1 214 7698,
Fax: +36 1 201 2680
E-mail: diamond@diamond-congress.hu

TOPICS

of the ISTA Seed Symposium,
Budapest, May, 2004

Theme
"Towards the future in seed production, evaluation and improvement"

Session 1:
Application of advanced technologies

Topics: Identification of GM, varietal identification; diagnostics in plant pathology.

Session 2:
Organic and conventional seed production

Topics: Seed crop management; harvest; processing; post-harvest technology; seed certification.

Session 3:
Viability and vigour: evaluation and impact

Topics: Methods of evaluation; influence of seed quality on seed performance and/or emergence, storage potential, responses to stress.

Session 4:
Seed systems in emerging and developing economies

Topics: Development and status of seed systems; seed quality management; evaluation of seed quality; role of informal and formal seed sector.

Session 5:
Seed lot hygiene

Topics: Weed contamination, seed-borne pathogens, pests, endophytes, phytosanitary standards.

Session 6:
Seed improvement

Topics: Dormancy breaking; seed sorting; priming; chemical and biological controls; pre-storage treatments; pelleting.

Session 7:
Physiological basis of seed quality

Topics: Seed development; desiccation; storage; molecular, cellular and biochemical aspects of germination.

Poster Session

Genetically Modified Crops

Current situation and some considerations regarding detection techniques

By Christoph Haldemann, ISTA GMO Task Force Member

Our technician with real-time PCR machines

The cultivation of genetically modified (GM) crops grew extensively all over the world in 2002. The estimated global area of GM crops for 2002 is 58.7 million hectares, grown by between 5.5 and 6.0 million farmers in sixteen countries - up from 5 million farmers and thirteen countries in 2001.

To put 58.7 million hectares into context, it is more than 5% the total land area of China or the US or almost two and half times the land area of the United Kingdom. The increase in area between 2001 and 2002 is 12%, equivalent to 6.1 million hectares. A sustained rate of annual growth of more than 10% per year has been achieved every year for the last six years, since their introduction in 1996. During the seven-year period 1996 to 2002, global area of transgenic crops increased 35-fold, from 1.7 million hectares in 1996 to 58.7 million hectares in 2002.

An increasing proportion of GM crops are grown in developing countries. More than one quarter (27%) of the global GM crop area of 58.7 million hectares in 2002, equivalent to about 16 million hectares, was grown in nine developing countries.

In 2002, four principal countries grew 99% of the global transgenic crop area. The USA grew 39.0 million hectares (66% of global total), followed by Argentina with 13.5 million hectares (23%) despite the economic situation, Canada 3.5 million hectares (6%), and China 2.1 million hectares (4%).

Globally, the principal GM crops were GM soybean occupying 36.5 million hectares in 2002 (62% of global area), followed by GM corn at 12.4 million hectares (21%), transgenic cotton at 6.8 million hectares (12%), and GM canola at 3 million hectares (5%).

During the six-year period from 1996 to 2002, herbicide tolerance has consistently been the dominant trait with Bt insect resistance, particularly the European Corn Borer, second. In 2002, herbicide tolerance, deployed in soybean, corn and cotton, occupied 75% or 44.2 million hectares of the global GM 58.7 million hectares, with 10.1 million hectares (17%) planted to Bt crops, and stacked genes for herbicide tolerance and insect resistance deployed in both cotton and corn occupying 8% or 4.4 million hectares of the global transgenic area in 2002.

In 2002 for the first time more than half of the world's population lived in countries where GM crops are approved and grown. There is cautious optimism that global area and the number of farmers planting GM crops will continue to increase in 2003. Currently the development of transgenic corn lines with new traits has become one of the main activities and research interests of the agro-industry (International Service for the Acquisition of Agri-biotech Applications, ISAAA).

The application (import, use and cultivation) of GMO crops as food, animal feed and seeds in Europe and Switzerland requires an authorization.

In Europe, the marketing and labelling of GMO's is regulated by the Novel Food Directive or by national Food and Feed Ordinances. The Swiss Ordinances, for example, are based on the detectability of the modified gene sequences.

Once approved they have to be labelled if the GMO's exceed a fixed threshold. For Switzerland these thresholds are: 0.5% for seeds, 1% for food, 3% for feed ingredients and 2% for mixed feeds. In addition, the EU has planned that additives and flavours containing GMO material have to be declared on the product's label, regardless of the amount used (Amended proposal for a REGULATION OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL ON GENETICALLY MODIFIED FOOD AND FEED, COM (2002) 559 final). Also large groups of consumers, especially in Europe are concerned about the use of GMO food. In order to comply with mandatory labelling regulations there is a strong need to have access not only to specific but also to quantitatively reliable methods, which are easy to handle.

Therefore, quality as well as safety of seed, food and feed is an issue of growing public concern. Public control institutions, private service laboratories, and commercial manufacturers see an increasing need to test for genetically modified organisms (GMO) in these matrices.

To fulfil the duty of the Swiss legislation, the Swiss Federal Research Station for Animal Production established its own GMO laboratories in 1997. As the GMO laboratories are accredited by ISO/IEC

17025, all methods have to be validated by a well-defined procedure.

Table 1 shows the methods to detect GMO's, available in our laboratories.
Basically we have two types of customers:
- External customers, e.g. seed importers, which send the samples straight to our laboratories.
- Internal customers, i.e. samples derived from the official seed and feed control in Switzerland.

Real-time multiplex PCR (Polymerase Chain Reaction)

Since in the last ISTA News Bulletin, No. 124, an article about the most common methods for detecting GMO's was published by Bettina Kahlert, the author needn't go into further detail in this issue. In addition to this article there is the real-time multiplex PCR. The fluorescence-based real-time PCR (rt-PCR) is widely used nowadays for the quantification of DNA and is a critical tool for basic research, molecular medicine and biotechnology. Assays are relatively easy to perform, capable of high throughput, and can combine high sensitivity with reliable specificity. The technology is evolving rapidly with the introduction of new enzymes, chemistries and instrumentation. The simultaneous amplification of more than one DNA sequence (in the same reaction tube) is called real-time multiplex PCR. This multiplex DNA-amplification is a very useful and powerful technique for the detection of GMO contaminations. All Swiss authorized GMO's contain a 35S-promotor insert. Therefore we use this technique in our laboratories as a quantitative screening test, i.e. to check quantitatively whether GMO's are contained in the sample. For this screening we established a system with which we can amplify the 35S-promotor gene and the invertase-gene respectively, the lectine-gene, simultaneously. For more detailed information visit our homepage: http://www.sar.admin.ch/rap/en/fodder/gvo_method_aug01.pdf.

If the customer wants to know what GM variety is responsible for a positive finding, the laboratory has to search selectively for the individual genetic engineering modifications. Thus, further examinations are necessary. Table 1 shows all specific GM varieties, which can be detected in our laboratory at the moment.

Quality management

Taking into account possible DNA degradation, due to food processing involving heat or chemical treatments, UV-radiation, etc, we compute the ratio of the amplified transgenic DNA to the amplified endogenous DNA. Errors of the endogenous DNA analysis can affect very much the final result of the transgenic content of the unknown sample. This ratio is calculated only for seed and feed ingredients. For mixed seeds we take only the 35S-promotor into account.

	Qualitive Methods	Quantitative Methods
Maize	BT11 (Novartis) Bt176 (Novartis) MON810 (Monsanto) T25 (Bayer AG) CBH 351 (Aventis) GA21 (Monsanto)	BT11 (Novartis) Bt176 (Novartis) MON810 (Monsanto) T25 (Bayer AG) Invertase (endogenous gene for maize)
Soybean		GTS 40-3-2 (Monsanto) Lectine (endogenous gene for soybean)
Canola	GT73/RT73	35S-Promotor

Table 1: Qualitative and quantitative methods to detect specifically different GM species, available in our laboratories.

To prevent errors and to ensure high quality of analysis we have to set stringent conditions for our results:
- If the result of the endogenous DNA is smaller than 1%, the final result will be given as: no DNA could be detected.
- If the result of the endogenous DNA is higher than 1% and the result of the transgenic DNA is higher than 0.02% the final result of the transgenic content is calculated by the ratio of the transgenic DNA to the total DNA.
- If the result of the endogenous DNA is higher than 1% and the result of the transgenic DNA is equal to or smaller than 0.02% the final result will be given as: result under the limit of quantification.

Further requirements to fulfil the high quality demands and to prevent contamination must be carried out by the GMO analysis in four spatial separated laboratories:

- For sample grinding
- For DNA extraction
- For master mix preparation
- For DNA amplification

Moreover we successfully take part in several different Proficiency Tests (PT), such as ISTA PT on GMO testing, GeMMA PT (Genetically Modified Material Analysis Scheme), Bipea (Bureau Interprofessionnel d'Etudes Analytiques).

Frequency of GMO analysis

In 2002 a total of more than 1500 samples were analysed by our laboratories. Approximately 10% of these samples consisted of seeds. As seeds the following species were found:
- soybeans
- corn
- canola
- sugar beet
- wheat
- tomato
- barley
- flax

The seeds were analysed qualitatively for the 35S-promotor and for the NOS-terminator (terminator of nopaline synthase gene derived from Agrobacterium tumefaciens). The 35S-promotor also exists in the cauliflower mosaic virus, hence, in case of positive results it must also be checked whether this might not possibly be the 35s-promotor from the virus, due to a contamination or a virus infection. For canola seeds, in addition to the previously mentioned were analysed for the GT73/RT73 event as well. For all investigated seeds there was no result to be rejected, i.e. there was no positive result.

Today's challenges for 'GMO Laboratories'

Since the analytical know-how regarding GMO detection is located in a rapidly developing field, one of the main goals for GMO laboratories is to efficiently develop and establish further methods for detecting newly designed GM crops launched on the world market. In this context access to certified reference material (CRM) is of paramount importance. Without CRM method calibration and method comparison between different laboratories becomes enormously difficult.

Last but not at least I would like to stress another relevant issue, the DNA extraction. Poor DNA quality, e.g. containing amplification inhibitors, such as polysaccha-

1st ISTA Proficiency Test on GMO Testing of *Zea mays* L.

By Bettina Kahlert, Proficiency Test Working Group Leader of ISTA GMO Task Force and
Michael Kruse, Rules Chapter Working Group Leader of ISTA GMO Task Force

The aim of the 1st Proficiency Test was to check the ability of the participating laboratories to detect the presence of GM seeds in samples of conventional seed of *Zea mays* L.

Each participating laboratory received a set of 30 maize samples. 12 samples were negative (no GM seeds added) and 18 samples were positive. Each sample contained about 300 seeds. The GMO content in the positive samples was about 1% (3 in 300). 6 out of the 18 samples were positive due to Mon810, 6 samples due to T25 and 6 samples due to both (1 seed from Mon810 and 2 seeds from T25).

A first communication of results was given in the ISTA News Bulletin 124.

43 laboratories from 20 countries worldwide (Fig. 1a), from governmental, company and other private seed testing laboratories participated (Fig. 1b).

Each laboratory reported for the individual sample whether this is a negative sample or a positive sample. So for a given sample the result reported by the laboratory can be either correct or false.

30 laboratories (70%) reported for all 30 samples correct results and 13 reported false results (30%, Fig. 2). On the laboratory basis more laboratories reported false negative results for T25 than for Mon810 (Fig. 3a). On the sample basis also the percentage of false negative results reported was higher for T25 than for Mon810 (Fig. 3b).

The following methods were used: 31 laboratories used qualitative PCR; 9 with false results. 4 laboratories used real time PCR; 1 with false results. 2 laboratories used qualitative and real time PCR combined with correct results. 2 laboratories used ELISA; 1 with false positive results. 2 laboratories used Bioassay, both with correct results. 1 laboratory used qualitative PCR and ELISA combined and reported false negative results. 1 laboratory used ELISA and Bioassay combined and reported false positive results.

The results of this proficiency test showed that about 70% of the participants were able to identify all 30 maize samples correctly. This is a clear indication of the high performance of the majority of the participants to perform both accurate GMO tests for the events T25 and Mon810 with a spiking level of 1% and accurate tests for negative samples. The remaining 30% showed the whole range from minor up to severe problems with GMO testing. This clearly indicates, the need for further training and standardisation efforts in these laboratories. Thus, in summary, the results provide a first estimate for the great potential of the performance based approach in the field of GMO testing as developed by ISTA in the GMO position paper. The ratio between laboratories without and with false results of 70:30 indicates that, the level of difficulty of the 1st Proficiency Test was appropriate, and shows the potential for further proficiency tests. In the view of ISTA, as an aim to provide uniform test results all over the world, 70% correct results is not sufficient and calls for action for improvement.

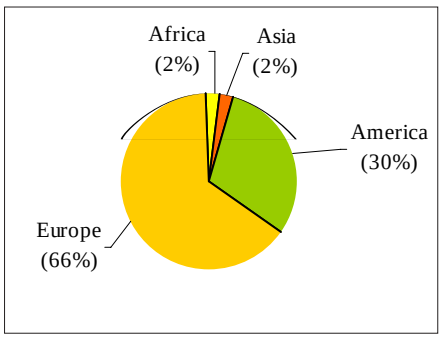


Figure 1a: Region of participating laboratories:

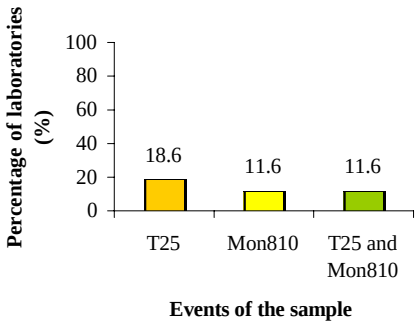


Figure 3a. Percentage of laboratories which reported false negative results with positive samples with T25, Mon810 and T25 and Mon810.

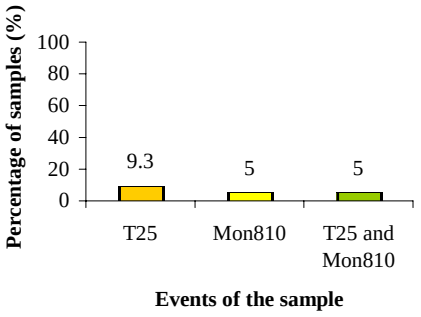


Figure 3b. Percentage of positive samples with T25, Mon810 and T25 and Mon810 which were reported as false negative results by the laboratories.

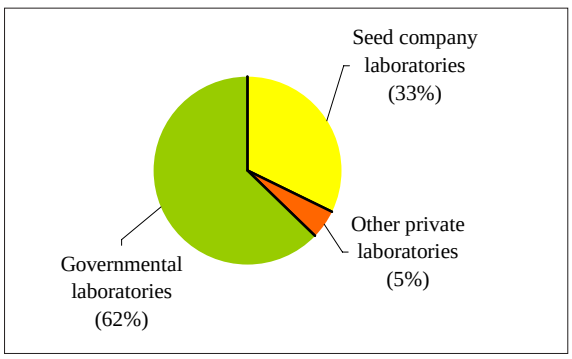


Figure 1b. Status of participating laboratories.

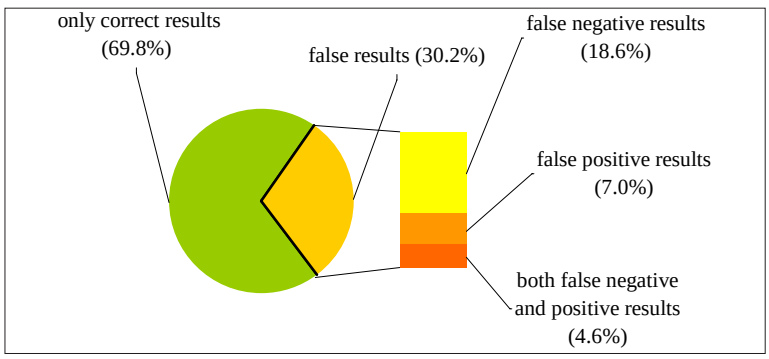


Figure 2. Percentage of laboratories reporting only correct results and false results.

Announcement of the 2nd ISTA GMO Proficiency Test

GMO Testing of *Zea mays* L.

By Michael Kruse, ISTA GMO Task Force Member
Bettina Kahlert, ISTA GMO Task Force Member

Aim

The aim of this 2nd ISTA Proficiency Test on GMO Testing is to check the ability of individual laboratories to detect and, on a voluntary basis, to quantify the presence of GM seeds in samples of conventional seed of *Zea mays* L.
The object of data analysis will not be to identify deviating laboratories but to compile the performances in the laboratories and to provide data for the laboratory´s internal performance data base.

Sample description

Each participating laboratory will receive 10 maize samples. They will be labelled only with an identification number provided by ISTA. Samples will contain approximately 3000 seeds. This number will not be exact as samples will be prepared on the basis of an average 1000 seeds weight. Some of the samples will be positive (i.e. contain GM seeds) and the others will be negative (i.e. contain no GM seeds). Laboratories will have to prepare the flours from seed samples.

Obligatory qualitative test

A qualitative result is requested. Laboratories can use the method they think appropriate for this test. The result for the qualitative test, i.e. a sample is positive or negative, must be sent back for each sample along with the sample identification number provided by ISTA. Participants are not expected to identify events in the positive samples.

Optional quantification of GMO in positive samples

On a voluntary basis laboratories can do a quantification of the GMO level in the positive samples by either a semi-quantitative test (sub-sampling strategy) or by a quantitative test.

Semi-quantitative test (sub-sampling strategy)
A semi-quantitative test using the sub-sampling strategy is optional to the participants. The participants shall report as a result of this test whether the GMO level in the test sample is above the level of 1% or not. Laboratories can use the method they think appropriate for this test. The result for the semi-quantitative test must be sent back for each sample along with the sample identification number by ISTA.

Quantitative test

The true quantitative test is also optional. This quantitative test is for checking the ability of the laboratories to quantify the GMO content in a sample. The participants shall report the GMO level of the test sample. Laboratories can use the method they think appropriate for this test. The result for the quantitative test must be sent back for each positive sample as estimates, along with the sample identification number by ISTA. Furthermore, a statement on the uncertainty of measurement should be reported. The procedures shall be clearly documented. The test procedures will not be evaluated.

Implementation

Participation fee

There is a participation fee for non ISTA members of 150 US$ for the 2nd ISTA Proficiency Test on GMO Testing which have to be paid as pre-requirement for receiving the samples The participation fee of ISTA Members is included in their ISTA membership fee.

Material Transfer Agreement

Participating laboratories in the 2nd ISTA Proficiency Test on GMO Testing are kindly requested to sign the Material Transfer Agreement with ISTA. It will be not possible to participate without signing it.

Shipping

The shipment you will receive will contain 10 samples, each of about 3000 seeds of Zea mays, in total about 10kg. The seeds are not treated. The samples are packed in hermetically sealed plastic bags and the shipment will be labelled as 'seeds for destructive laboratory tests'. If necessary, a phytosanitary certificate will be attached.

Time schedule

1. Registration February 05, 2003 to April 18, 2003
2. Final submission of results to the ISTA Secretariat May 16, 2003
3. Presentation of the overall results and conclusions July 01, 2003 (ISTA Extraordinary Meeting, Zürich)

Thank you for your interest, we are looking forward to a successful proficiency test. ■

Genetically Modified Varieties and the Seed Industry

By Bernard Le Buanec, ISF Secretary General

Genetic engineering started in the early 1970s and was at that time limited to bacteria.

But rapidly the technology was extended to the plant kingdom and the first transgenic plants, tobacco plants, were published in 1983. Since then, the evolution has been very fast and the transgenic plant varieties, known as GMOs, were grown on more than 60 million hectares in the world in 2002.

GMOs today are in the "heart" of a passionate debate. When speaking about GMOs, there are in fact two different aspects that are often confused: one is the development of genetically modified (GM) varieties and their commercialization and use, the other one is the adventitious presence of GM material in non-GM varieties.

1. Development of GM varieties

1.1. Historical background

The history of plant transformation began with the discoveries made by phytopathologists working on soil bacteria responsible for crown gall and "hairy root" syndrome in the 1960s[1]. In 1974 , it was demonstrated that these "natural" transformations were due to the transfer of plasmids from bacteria to the plant[2]. Using these plasmids, the first stable expression of a "foreign" gene in a plant was demonstrated for the first time in tobacco in 1983[3]. It was the first GM plant.

In 1987 the first field trials were planted in France and USA, and in 1994 the first authorization for commercial production of a transgenic variety, a Tomato, was obtained in the USA.

1.2 The present situation of transgenic crops in the world

Since 1994 the acreage of transgenic varieties has increased drastically, the technology being probably the one adopted the more rapidly by farmers in the recent history of agriculture. The evolution is as follows[4],[5].

Global Area of Transgenic Crops, from 1996 to 2002

Year	Million hectares
1996	1.7
1997	11.0
1998	27.8
1999	39.9
2000	45.2
2001	54.2
2002	60.7

During the seven-year period 1996 to 2002, the global area of transgenic crops increased by 35 fold, reflecting the growing acceptance of transgenic crops by farmers in both industrial and developing countries.

By country, the situation is as follows:

Global Area of transgenic crops from 1998 to 2002 (millions of hectares)

Country	2000	%	2001	%	2002	%
USA	30.3	67	35.7	66	39.0	64
Argentina	10.0	22	11.8	22	13.5	22
Canada	3.0	7	3.2	6	3.5	6
China	0.5	1	1.5	3	2.1	3
Brazil	1.0	2	1.6	3	2.0	3
South Africa	0.2	<1	0.2	<1	0.3	<1
Australia	0.2	<1	0.2	<1	0.1	<1
India	--	--	--	--	<0.1	<1
Romania	<0.1	<1	<0.1	<1	<0.1	<1
Spain	<0.1	<1	<0.1	<1	<0.1	<1
Uruguay	<0.1	<1	<0.1	<1	<0.1	<1
Mexico	<0.1	<1	<0.1	<1	<0.1	<1
Bulgaria	<0.1	<1	<0.1	<1	<0.1	<1
Indonesia	--	--	<0.1	<1	<0.1	<1
Colombia	--	--	--	--	<0.1	<1
Honduras	--	--	--	--	<0.1	<1
Germany	--	--	<0.1	<1	<0.1	<1
France	<0.1	<1	--	--	--	--
Total	**45.2**	**100**	**54.2**	**100**	**60.7**	**100**

By crop, the situation is as follows:

Global area of transgenic crops from 2000 to 2002 (millions of hectares)

Crop	2000	%	2001	%	2002	%
Soybean	26.8	59	34.9	64	38.5	63
Maize	10.3	23	9.8	18	12.4	21
Cotton	5.3	12	6.8	13	6.8	11
Canola	2.8	6	2.7	5	3.0	5
Squash	<0.1	<1	<0.1	<1	<0.1	<1
Papaya	<0.1	<1	<0.1	<1	<0.1	<1
Potato	<0.1	<1	--	--	--	--
Total	**45.2**	**100**	**54.2**	**100**	**60.7**	**100**

Examples of Seed Prices for GMO's and "conventional" Crops

Cotton		**Bt**	**Conventional**	**Technology fee**	
Argentina	20 kg	100 US$	30	70	70%
India	450 gr	1400 Roupies	400	1000	71%
Maize		**Bt**	**Conventional**	**Technology fee**	
Argentina	20 kg	100 US$	80	20	20%
USA	80 M units	103 US$	80	23	22%
Soybean		**RR**	**Conventional**	**Technology fee**	
Argentina	50 kg	20 US$	17	3	15%
USA	50 pounds	22 US$	13	9	41%

In terms of value, the market of GM varieties seed was US$ 3.0 billion in 2000, increasing to US$ 3.8 billion in 2001. It is estimated at US$ 4.25 billion in 2002, representing almost 15 % of the 30 billion value of the global commercial seed market. The market value of the seed of GM varieties is based on the sale price of the seed, plus the technology fees that apply[6].

1.3 Regulatory Issues

1.3.1 Regulations at international level

Discussions are going on at international level on regulation of GMO and products issued from GMO in several fora as Codex Alimentarius, OECD Seed Schemes, the Convention on Biological Diversity (CBD), but except the Biosafety protocol, which is a protocol to the CBD, at present no agreement has been reached.

In summary, the Protocol on Biosafety, known as the Cartagena Protocol, adopted in February 2000, states that transboundary movement of LMO (Living Modified Organisms) with the objective of intentional introduction into the environment of the importing country must be subject to the advance informed agreement of that importing country.

Are excluded from that obligation:

- LMOs which are pharmaceuticals for humans that are addressed by other international agreements;
- LMOs in transit or for contained use;
- LMOs intended for direct use as food or feed, or for processing.

In fact, in the plant domain, the protocol will apply almost exclusively to seed for planting.

In addition, LMOs which are subject to intentional transboundary movement within the scope of the Cartagena Protocol must fulfill special handling, transport, packaging and identification:

- LMOs that are intended for food, feed or for processing must be clearly identified as "may contain" LMOs.
- LMOs that are intended for intentional introduction into the environment must be clearly identified as such. The accompanying documentation must specify the identity and relevant traits and/or characteristics, any requirements for the safe handling, storage, transport and use, the contact point for further information and, as appropriate, the name and address of the importer and exporter; and contain a declaration that the movement is in conformity with the requirements of the Cartagena Protocol applicable to the exporter. The practicalities of these requirements are presently under discussion, in which ISF is involved. The Protocol will enter into force when ratified by 50 countries. Today, 44 have ratified and the entry into force will likely be in 2004, impacting on seed companies for their seed shipment, in particular for the winter nurseries.

1.3.2 Regulations at national level

The situations are very diverse from country to country, not in terms of the technical approach of safety assessment, but in terms of general regulations and acceptability.

As regards safety assessment, all the countries where appropriate laws and committees do exist, the main focus is on environment and food safety, and for food safety, more particularly on possible allergenicity. The process is always very comprehensive. It is worth noting that the procedures are very efficient as, since 1994, no accident has been documented, although LMOs/GMOs are grown on tens of millions of hectares every year and products derived from GMOs are eaten by hundreds of millions of people every day. The disadvantage is that the safety assessment is extremely costly, between 1 and 2 million dollars for crop/trait. The consequences are that such costs exclude de facto from development small and medium size companies and small crops. In fact we are here in a "Catch 22" situation: the opponents to the technology claim that only large multinational companies are developing new traits for few major crops but, by their request to add new tests in the safety assessment procedures they are reinforcing that trend. When enough experience is gained in those issues, and we have now 20 years of experience, those constraints should be taken into account when designing and implementing the safety assessment procedure.

The general regulations on GMOs differ drastically form country to country, from a total ban, as in Algeria[7], to a complete liberalization after the safety assessment has been proved satisfactory, as in USA or Argentina, by moratorium or de facto moratorium, such as in the European Union. It seems however that there is a global trend to the acceptability of GMOs at world level. The European Union has put in place in 2002 legal instruments that should lead to the lift of the de facto moratorium even if some European countries are still reluctant; most of the African countries are now more interested in the transfer of technology than in the ban of the GMOs[7] and much of Asia is rushing forward with the development and cultivation of GMOs[8].

However, the development of compulsory labeling of GMOs in several countries lead to a very difficult problem facing the Seed Industry: the adventitious presence of GM material in non-GM varieties.

The Adventitious Presence of GM material in non-GM varieties is continued on page 14.

2. The Adventitious Presence of GM material in non-GM varieties

2.1 Background

The adventitious presence of GM material in seed, food and feed is the unintended occurrence of plant material from crops improved through biotechnology. It is of course the logical and unavoidable consequence of the development of GMOs at world level. That adventitious presence may occur through natural pollen flow or from commingling that occurs in the production/distribution system.

Some countries, that are labeling GMO food and food ingredients, have defined thresholds above which the products should be labeled as GMOs. For instance in Europe the trigger point is 1%, in Japan 5% and in Brazil 4%. (Incidentally it is interesting noting the huge variations as regards the thresholds chosen, showing clearly that they are not based on scientific information but on political posturing). As early as 1999, FIS and ASSINSEL realized that labeling of GMOs and consequent demand for separation of GM and non GM products would have impact on the seed trade. During their Melbourne Congress in June 1999, the following motion was adopted:

FIS and ASSINSEL Urge Countries to Adopt Practical Seed GMO Thresholds

"FIS and ASSINSEL consider that when the environmental and nutritional safety of GMOs and GMO products have been positively assessed, there is no scientific reason to separate these products from the non-GMO ones.

However, for various reasons, some markets are demanding separation of GMO and non-GMO products.

Whereas there is a link between the quality of seed and products derived therefrom; and

Whereas new genetic and analytical technologies (e.g., PCR amplification of DNA) have made it possible to detect minute amounts of all off-types; and

Whereas the world's seed industry has established and observes standards and management practices that result in seed products of consistently high quality in which the presence of off-types is limited to an agreed upon standard;

FIS and ASSINSEL support seed quality standards with practical, defined seed GMO thresholds for adventitious genetic off-types from any source, that are consistent with current seed production practices and industry standards on a crop by crop basis."

In August 1999, FIS launched an international initiative to deal with this issue, which was known at the end of that year as the International Seed Network Initiative, ISNI, with FIS, OECD, ISTA and AOSA. That initiative had three main objectives:

- Review the production processes to minimize the adventitious presence by pollen flow and commingling and if necessary to design new quality insurance charts.
- Establish laboratory's standards for assessing the presence of GM material in non GM product.
- Fix an experimental threshold of 1%, globally recognized.

Unfortunately, it was not possible to reach an agreement on that proposal and after 3 years of discussions, the principle of such an experiment has been abandoned. Discussions are continuing with a less exhaustive approach on possible thresholds and seed testing. They have shown that the issue was technically complex and politically sensitive.

The Adventitious Presence issue can be divided in 3 main categories:

1) Events that have been approved for feed.
2) Events that have been/will be approved for pharmaceutical uses.
3) Events that have been approved for food.

Incidentally it is interesting noting the huge variations as regards the thresholds chosen above which the products should be labeled GMOs, showing clearly that they are not based on scientific information but on political posturing. However, the lack of harmonization at international level is creating new trade barriers. A science-based approach should facilitate the development of harmonized international regulations.

There is an agreement that Adventitious Presence of events of the first two categories should not be accepted at all. It is very unlikely that, after the Star Link experience, companies will put on the market food crops events approved for feed only and the risk of A.P. is almost virtual.

The events for pharmaceutical uses deserve a special attention and the industry is taking the matter extremely seriously. There are discussions at the moment within the agro-food industry on the possible ban of "pharmaceutical events" in food crops.

As regards the events that have been approved for food, three main subcategories may be established:

1) The adventitious presence of GM seed, of events that are domestically approved.

2) The adventitious presence of GM seed, of events that are not domestically approved but have been approved in another country that employs OECD safety assessment process.

3) The adventitious presence of GM seed, of events which are still in the research stage and have received approval for field tests in any country member of the OECD Seed Schemes.

Categories 1 and 2 are of primary importance with regard to adventitious presence. However, adventitious presence of event of category 3 also need to be taken into account, even if it is extremely unlikely.

Where are we at the moment ?

2.2 Thresholds

2.2.1 At international level

The discussions are going on at OECD level. The position of ISF is that zero tolerance is unachievable in practice and that a 1% threshold for event approved, including approved field trials, in any of the member countries of the OECD Seed Schemes is a reason-

able transitional solution taking into account seed production realities and the absence of documented risks. Despite several meetings, no agreement has been reached for two main reasons:

- All the countries involved in the discussion are reluctant to include events which have not been approved by themselves. Of course that position is much less damaging in countries where many events are approved like for instance North America and Japan than in countries like Europe where very few events are approved due to the current de facto moratorium.

- There is no agreement on the threshold level, some countries supporting ISF position, as USA, Argentina, Canada, Australia, …, some asking for lower thresholds, 0%, 0.3% or 0.5%, as the European Union.

It is very unlikely that an agreement can be reached in the near future.

2.2.2 At national level

Few regulations have been put in place. In Switzerland, the provisional level is 0.5% for events approved in the country and in the European Union. It is an interesting step towards the acceptance of decision from other countries but the threshold is rather low. In Argentina, the threshold is 1%. In New Zealand, for sweet corn it is 0%, tested according to an official protocol.

In the European Union the existing official proposal states that:

- Genetically modified seed not having authorization for the placing on the market shall not be present
- Genetically modified seed having received authorization shall not exceed the standards laid down for varietal purity and, in any case, not more than:
  - 0.3% for seed of a cross-pollinating species or variety, other than maize.
  - 0.5% for seed of maize.
  - 0.5% for seed of a self-pollinating species or variety other than field peas and soybean.
  - 0.7% for seed of field peas, or soybean.

Internal discussions among countries member of the European Union are not yet completed.

ISF stresses that in fixing thresholds countries have to take into account the situation where almost all the crops would be GMOs as in some areas in the world at the moment. In that case a practical threshold should be the average percentage of off-types which are present at the moment in commercialized seed.

2.3 Seed testing

Seed testing comprises two different parts: seed sampling and detection of presence of GM material. Scientifically it should not be too difficult to resolve that problem. However, from a practical and economical point of view, detection methods remain a major issue. This point will have to be taken into consideration when threshold and detection methods are proposed, while maintaining reliable methods and providing valuable information.

- Seed Sampling

Several organizations are working on this issue, including the ISTA GMO Task Force, AFNOR in France, CEN and ISO and probably others. At the moment there is no rule globally adopted but the work done by CEN and ISO should be finalized shortly.

- Detection

A variety of methods, both qualitative and quantitative, are currently used in government, company and third party labs. Unfortunately, for many of these methods overall performance parameters have not yet been established and well-characterized reference material, with documented purity measurements, are in general not available for validation studies.

Several organizations are working on detection protocol such as, for example, GIPSA in USA and JRC in Europe.

For seed, a first ring test was run by ISF in the year 2000, but due to the low level of participation, the results were not significant.

ISTA, in the year 2000, has established a "GMO Task Force" with the participation of other actors of the international seed chain: ISF, AOSA and ISST.

In 2001, ISTA adopted a "Strategy regarding Methods for the Detection, Identification and Quantification of Genetically Modified Seeds in Conventional Seed Lots"[9], with in particular a new performance-based approach. In the frame of this new approach, proficiency tests are performed. In 2002, 43 test series by 41 laboratories on the traits T25 and Mon810 have shows that progress has to be made, as 13 series reported false results, i.e. 30%. A new test should be done in 2003.

It is important that only results obtained by laboratories duly recognized/accredited by national authorities be taken into consideration for regulatory purposes.

3. Conclusion

GMOs are expanding rapidly at a global level, with differences between countries. This development is creating new opportunities for the seed industry.

However, the lack of harmonization at international level is creating new trade barriers and, in particular, the possible adventitious presence of GM material in non-GM seed has already caused some disruptions in the international seed trade. A science-based approach should facilitate the development of harmonized international regulations.

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APSA Marches Forward

The Asia & Pacific Seed Association

By J.S. Sindhu, APSA Director

In the Asia and Pacific region, agriculture is the mainstay of the economies in the majority of countries. It is a paradox that as to date a big gap still exists between demand and supply of quality seeds in the entire region. There is also an urgent need to improve seed quality. In this effort, assistance of ISTA will undoubtedly be of great significance.

The Asia and Pacific Association (APSA) is the largest regional seed association in the world. Active membership of over three hundred provide enough strength but diverse agricultural, socio-economic, and environmental situations prevailing in the 30 member countries offer several challenges. While the seed industry is quite organized in some developed countries such as Australia, New Zealand and Japan, it is at various stages of development in others It is important to understand that Asia-Pacific region shares 23 percent of global land area and nearly 56.6 percent of world population. Countries with large land area include China, Australia, India, Indonesia, Iran and Mongolia.

China, Japan, India and Australia share substantially world commercial seed market, while the seed industry is developing fast in other countries of the region. During past few years, APSA helped the seed industry considerably in understanding market information and arranged trainings and visits for its members. This has helped privatization and the industry is slowly entering the mainstream of global seed business.

APSA developed technical and market related information and a number of such reports are available with us. These include the following:

- Technical reports on new technologies.
- Country reports of the seed industry in the region.
- Information on seed production, conditioning, storage, quality assurance and marketing.
- Information on IPRs and other related issues.
- Seed import/export statistics for different countries in the region.

The above information is shared with our members and a part of it is also published in our journal - *Asian Seed and Planting Material* and can also be accessed through our website: http://www.apsaseed.com

APSA has all the national seed associations in the region as its members and we interact with them regularly. In addition, we also interact on issues of mutual interest with other regional seed associations such as AFSTA, ASTA, and FELAS.

The main issues facing the seed industry in the region include:

- Increasingly difficult access to available germplasm due to uncertainty on IPR issues.
- Phytosanitary regulations being used as non-transfer barriers, often clogging free flow of seed at transnational level.
- Seed quality assurance need to be improved and ISTA 's assistance is crucial.
- PVP/patents are not yet in place in several countries as a result of which seed trade is not expanding at the desired pace.
- Inadequate investments in agbiotech research and limited information on transgenics may be critical for the adoption of GM seeds.

In the Asia and Pacific region, agriculture is the mainstay of the economies in the majority of countries. It is a paradox that as to date a big gap still exists between demand and supply of quality seeds in the entire region. There is also an urgent need to improve seed quality. In this effort, assistance of ISTA will undoubtedly be of great significance. ■

The Asia and Pacific Seed Association

Kasetsart Post Office
P.O. Box 1030
Bangkok 10903
TH-Thailand

Phone +66 2 940 5464
Fax +66 2 940 5467

apsa@apsaseed.com

The International Plant Protection Convention (IPPC)

The Harmonisation Of International Phytosanitary Measures Through The Use Of ISPMs And The Need For Capacity Building Under The IPPC

By D.C. Nowell, and R.L. Robert, IPPC Secretariat

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Abstract

The International Plant Protection Convention (IPPC) is a legally binding international agreement deposited with FAO. The purpose of the Convention is "*to secure common and effective action to prevent the spread and introduction of pests of plants and plant products, and to promote appropriate measures for their control*". Although the IPPC clearly has strong relevance to the regulation of trade, the Convention is not limited in this respect. International cooperation in many forms of plant protection may fall within the scope of the Convention. Likewise, plants are not limited to cultivated plants and protection is not limited to direct damage from pests. Therefore, the scope of the Convention extends to the protection of natural flora and includes indirect harm or damage from pests such as weeds. The 1997 revision of the IPPC, modernised the Convention and brought it in line with the WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement). This revision process included the establishment of the Secretariat for the IPPC (based in FAO), the Commission on Phytosanitary Measures (CPM), and the formalisation of the standard setting process. The SPS Agreement specifically nominated the IPPC as the formal body responsible for the setting of International Standards for Phytosanitary Measures (ISPMs). ISPMs are used to harmonise phytosanitary measures that will facilitate trade and minimise unjustified phytosanitary barriers to trade. Should an international standard not be available, phytosanitary measures must be based on a pest risk analysis.

The entry into force of the SPS Agreement and the reorganisation and changes in emphasis in the New Revised Text of the IPPC (1997), have resulted in countries needing to significantly review national legislation and phytosanitary capacity to meet domestic and international obligations. Given the large discrepancies between the developed and developing countries developing countries are in need of substantial capacity building, including the development of institutional capacity, legislative frameworks, and technical expertise and capacity. A component of this includes the use of and training in the understanding and implementation of international standards for phytosanitary measures. In this context, the ISTA seed health standards could play a more meaningful role in future, if a way is found to formally include them in the international process. Expertise within the framework of ISTA could also be utilized more effectively in this capacity building process.

Historical Background

The International Plant Protection Convention (IPPC) is a multilateral treaty for cooperation in plant protection that had its beginnings with the agreement by twelve countries to regulatory measures for grapevines under the Phylloxera Convention of Berne in 1881. This represented the first efforts at formalizing international cooperation in plant protection and led to the recognition of the need to address other plant pests and enlist cooperation among all countries.

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The first text of an international convention with broader objectives was drafted at the International Conference for Plant Protection held in Rome in 1929. After a long lapse due to a world war, the draft text was again brought to the attention of governments. This time, the forum was the young FAO in its Third and Fourth Sessions of Conference in 1947 and 1948 respectively. In 1951, the Sixth Session of FAO Conference adopted the Convention and it was deposited with the Director General of the Organization shortly thereafter. The Convention first came into force in 1952 after ratification by three signatory governments; Ceylon, Spain, and Chile.

Amendments to the IPPC were proposed in 1973. After a series of consultations, members agreed upon modifications that involved updating terminology and describing certain changes in the model phytosanitary certificates. The amendments were adopted by FAO in 1979. The Revised Text of the Convention came into force in 1991 following acceptance of the amendments by two-thirds of the contracting parties.

In 1986, the landscape began to change significantly as the General Agreement on Tariffs and Trade (GATT) entered into an eighth round of multilateral trade negotiations known as the Uruguay Round. Prior to the conclusion of these negotiations in 1993, it was clear to IPPC Members and

FAO that the IPPC would have a prominent position in the Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement). The role envisioned for the IPPC was to encourage international harmonization and elaborate international standards to help ensure that phytosanitary measures were not used as unjustified barriers to trade.

In response, FAO established a Secretariat for the IPPC in 1992, followed by the formation of the Committee of Experts on Phytosanitary Measures (CEPM) in 1993. The Secretariat immediately began an ambitious program of standard setting. At the same time, interest mounted among IPPC Members to amend the Convention to more accurately reflect the contemporary role of the Convention, particularly with respect to the relationship of the Convention to the SPS Agreement.

Negotiations for revision started in 1995 and were finalized in November, 1997 when the 29th Session of FAO Conference approved the New Revised Text (NRT) of the IPPC. The amended IPPC now requires ratification by two-thirds of its contracting parties to come into force.

The IPPC is an international treaty, deposited with FAO and administered by FAO but implemented through the cooperation of member governments and Regional Plant Protection Organizations (RPPOs). The IPPC currently has 117 contracting parties (members), and 40 countries have already adhered or accepted the NRT of the IPPC. Contracting parties to the IPPC includes most major trading partners except China and the European Community (the individual Members of the European Community are all Members and the EC will be eligible for membership when the NRT of the IPPC comes into force.

Objectives and Scope

The purpose of the Convention is "to secure common and effective action to prevent the spread and introduction of pests of plants and plant products, and to promote appropriate measures for their control".

Although the IPPC clearly has strong relevance to the regulation of trade, the Convention is not limited in this respect. International cooperation in many forms of plant protection may fall within the scope of the Convention. Likewise, plants are not limited to cultivated plants and protection is not limited to direct damage from pests. Therefore, the scope of the Convention extends to the protection of natural flora and includes indirect harm or damage from pests such as weeds.

The role of the Convention with respect to trade has changed significantly in recent years as reflected in the substantial amendments found in the New Revised Text approved in 1997. In addition to describing the fundamental elements of national plant protection organizations, the IPPC is now also an active organization strongly involved with international standard setting.

Organizational Structure

From 1951 to 1992, the IPPC existed as an international agreement administered through FAO. Beginning in 1986, activities under the Convention began to be coordinated through annual Technical Consultations with RPPOs organized by FAO.

In 1992, FAO established a Secretariat for the IPPC followed by the formation of the Committee of Experts on Phytosanitary Measures (CEPM) in 1993. The Secretariat is charged specifically with the coordination of the work programme of the IPPC, particularly the elaboration of International Standards for Phytosanitary Measures (ISPMs). The work was supported by the CEPM, a group of international experts which met annually to review and comment on the suitability of documents prepared by the Secretariat. This expert group has now been superseded by the Interim Standards Committee which meets biannually for the same purpose.

The ISTA seed health standards could play a more meaningful role in future, if a way is found to formally include them in the international process.

The New Revised Text of the Convention makes provision for the formation of a Commission on Phytosanitary Measures. In adopting the New Revised Text, the 29th Session of FAO Conference approved several interim measures, including the establishment of an Interim Commission on Phytosanitary Measures (ICPM) until the amendments come into force and a permanent Commission is in place. Because the ICPM is authorized and organized under provisions of the FAO Constitution and Basic Texts, it is open to all FAO Members. When the amended Convention comes into force, the permanent Commission will be comprised of the contracting parties of the IPPC.

Key National Obligations

In terms of the IPPC, National Plant Protection Organizations (NPPOs) must be established and administered by contracting parties. Additional, key obligations are the requirement to nominate an identify official contact point, conduct treatments and certify exports, participate in the development of, and observe / adhere to adopted, ISPMs. Technical assistance / capacity building is also a basic need in the IPPC and countries are encouraged to participate within their capacity and resources.

Key Activities Within the IPPC

The key activities within the IPPC framework are:
i) the international phytosanitary standard setting (ISPMs) process;
ii) the development of pest risk analysis for various classes of pests e.g. quarantine pests and regulated non-quarantine pests;
iii) the establishment of a technical dispute resolution process;
iv) the facilitation of official phytosanitary information sharing (including legislative / regulatory information, conduct surveillance of/for pests, ports of entry, NPPO structure, pest distribution, pest lists, pest outbreaks, official national contact points, regulations, non-compliance, disputes, and obligations under 1997 IPPC); and
v) providing and facilitating technical assistance / capacity building given the large needs of developing countries given the 1997 revision of the IPPC.

The International Standard Setting Process

Draft standards may be developed by the Secretariat or submitted to the Secretariat from any source. Nomination of topics for ISPM development may come from the ICPM, NPPOs, RPPOs, the IPPC Secretariat, or from another source via the IPPC Secretariat. The topic are finalised, and priorities established, at the annual meeting of the ICPM. In general, standards have their origin in national or regional initiatives, and/or are drafted by expert groups organized by the Secretariat based on the topics and priorities identified by the ICPM and specifications agreed by the Standards Committee (SC). Draft standards that have been reviewed and accepted by the SC are circulated to IPPC member governments

for consultation before being finalization of the draft standard by the SC for submission to the Commission for possible adoption.
IPPC standards fall into three categories: reference standards; concept standards; and specific standards. The IPPC has produced primarily reference and concept standards that provide the foundation for specific standards that may follow. The number and frequency of standards under development has a direct relationship to the resources available to the Secretariat and the technical complexity of the issues being addressed.

ISPMs completed and the year of their adoption:

No. 1: Principles of plant quarantine as related to international trade, 1995
No. 2: Guidelines for pest risk analysis, 1995
No. 3: Code of conduct for the import and release of exotic biological control agents, 1995
No. 4: Requirements for the establishment of pest free areas, 1995
No. 5: Glossary of Phytosanitary Terms, 1996 (revised 2002)
No. 6: Guidelines for surveillance, 1997
No. 7: Export certification system, 1997
No. 8: Determination of pest status in an area, 1998
No. 9: Guidelines for pest eradication programmes, 1998
No. 10: Requirements for the establishment of pest free places of production and pest free production sites, 1999
No. 11: Pest risk analysis for quarantine pests, 2001
No. 12: Guidelines for phytosanitary certificates, 2001
No. 13: Guidelines for the notification of non-compliance and emergency action, 2001
No. 14: The use of integrated measures in a systems approach for pest risk management, 2002
No. 15: Guidelines for regulating wood packaging in international trade, 2002
No. 16: Regulated non-quarantine pests: concept and application, 2002
No. 17: Pest reporting, 2002

Possible role for ISTA in the ISPM standard setting process

In future it is possible that ISTA may be able to play a role in this formal IPPC international standards setting process. However, any procedure or information submitted, through the process outlined above, would be submitted to the normal full review process of the IPPC i.e. the fact that it may have been approved by ISTA members will have no bearing on the process or procedure within the IPPC standard setting process. This also means that ISTA may want to consider ways of increased official government involvement and possibly expend their membership to facilitate this process. In addition, ISTA probably needs to find ways of improving cooperation and coordination with RPPOs. The benefits to ISTA would be international acceptance as an ISPM that has legal status within the WTO process.

To start the process of cooperation it would also be advisable to concentrate of pests of quarantine importance (e.g. *Erwinia stewartii*) and regulated non-quarantine pests (e.g. *Xanthomonas* in beans). These would be of particular interest to the IPPC contracting parties and are more likely to be supported by RPPOs.

Capacity building

Capacity building is crucial to the implementation of the IPPC, particularly for developing countries. For this reason there is a large on-going technical assistance programme from FAO and others donors that covers:
i) infrastructure and institutionalization (including policy and legislation, training, implementation of the ISPMs, and equipment / facilities);
ii) dispute avoidance and resolution; and
iii) pest emergencies.

Opportunities for ISTA in Capacity Building

There are also potential opportunities for ISTA in the area of capacity building. This includes possibly participating in the implementation of relevant specific standards, and in relevant training programmes developed by donor agencies. In addition, the ISTA community could act as a valuable source of technical experts and advice in relevant projects. However, for this to materialise there is a greater need for communication, improved networks and feedback, and a possibly increased participation in projects and project development. This is a goal that our oganizations can work towards.

Conclusions

In conclusion it can be noted that the IPPC is the tool for harmonisation of international phytosanitary measures through the use of ISPMs, there are significant capacity building needs in developing countries; and ISTA could probably play a more meaningful role in both these areas if they desired. However, should this be desirable, such partnerships, cooperation and coordination need to be developed carefully in the near future.

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IPPC Secretariat,
Plant Protection Service,
Plant Protection and Production Division,
Agriculture Department,
Food and Agriculture Organization of the United Nations,

vialle Delle Terme di Caracalla,
00100 Rome, Italy.

Correspondence:
Dave.Nowell@fao.org

Seed Health Testing

The State of the Art of Seed Health Testing

By Jim Sheppard, ISTA Plant Disease Committee Chair

Diagnostics in research and in routine seed health testing have similar objectives but health tests must be completed within a limited time span and standardisation is necessary to achieve results, which are consistent when applied in practice.

Seed is the basic unit of production for the world's food crop. In recent years seed has become an international commodity used to exchange germplasm around the world. Seed is, however, also an efficient means of introducing plant pathogens into new areas as well as providing a means of survival for pathogens from one cropping season to another. Testing seeds for germination and purity as a measure of seed quality has been a universal practice since the first seed testing station was established by Nobbe in Thrant Saxony in 1869. Nobbe mentions smut balls and sclerotia in connection with seed in his 1876 publication Samenkunde but does not describe any method for their detection except visual examination of the seed. The publication of the Manual for Determination of Seedborne Diseases in 1938 by Dr. L. C. Doyer was the first to describe reproducible methods for detection and identification of fungi, bacteria, nematodes and insects associated with seed.

Today seed health testing is routinely carried out in most countries for domestic seed certification, quality assessment and plant quarantine. Methods for seed health testing often vary from one laboratory to another. In 1957 the Plant Disease Committee established a comparative seed health testing program aimed at standardizing techniques for the detection of seedborne pathogens. The basic principle of the comparative testing program involved the distribution of uniform samples to a number of laboratories working independently followed by annual workshops in which the results were compared. The objective was to develop and standardize simple methods suitable for application on seed lots moving internationally.

Diagnostics in research and in routine seed health testing have similar objectives but health tests must be completed within a limited time span and standardisation is necessary to achieve results, which are consistent when applied in practice. There are six main requirements for seed health tests.

1. Specificity: the ability to distinguish the target pathogen from all organisms likely to occur on seeds from field or store, i.e. to avoid false positives.
2. Sensitivity: the ability to detect organisms, which are potentially significant in field crops at a low incidence in seed stocks.
3. Speed: in some cases, small concessions to accuracy may be necessary to ensure rapid results, but such results should be followed by more definite testing.
4. Simplicity: the methodology should minimize the number of stages to reduce room for error and to enable tests to be performed by not necessarily highly qualified staff.
5. Cost effectiveness: test costs should form part of acceptable production margins for each crop.
6. Reliability: test methods must be sufficiently robust so that results are repeatable within and between samples of the same stock regardless of who performs the test (within the bounds of statistical probability and sample variation).

Historically seed health tests have been classified into four distinct groups based on the general techniques used to observe the target pathogen these are (1) Direct Inspection of dry seed or examination of suspensions obtained from washings of the seed; (2) Incubation tests, Blotter or Agar Plate; (3) Staining and examination of the embryo; and (4) Immunoassays and Molecular Techniques.

Direct Inspection

Seed samples may be examined dry for the presence of ergots, other sclerotia and smut balls without or with a stereomicroscope. The sample may be immersed in water or another liquid to make fungal fruiting bodies, for example pycnidia, or symptoms more visible or to encourage the liberation of spores. After immersion, the seeds are examined by means of a stereomicroscope. Seeds may be immersed in water containing a wetting agent or in alcohol and shaken to remove fungal spores, hyphae, etc. attached to or carried with the seeds. Excess liquid is then removed and the extract is examined at a higher magnification using a compound microscope. Although seed inspection methods are useful for determining the incidence of easily visible and relatively superficial fungi or fungal bodies, they give no indication of their viability.

Incubation Tests: Agar Plate

The agar test gives an indication of the viable inoculum present in an infected seed sample. This is done by placing seeds onto sterile agar, potato dextrose or malt agars are most commonly used to encourage the growth of seedborne fungi. Plates are incubated at 20°C in the dark for 7 days when the characteristic growth form (mycelium) of the fungus can be identified by eye or using a low or high power microscope. Near-ultraviolet light (NUV) may be used during incubation to encourage the development of fruiting bodies. Seeds are spaced on the Petri dishes to avoid cross-contamination. There are many variations of the agar test. Acidic agars may be used to reduce bacterial contaminants agars may be made semi-selective by the addition of specific chemicals and/or antibiotics and/or fungicides.

Incubation Tests: Blotter Tests

The blotter test gives an indication of the infection of the seed, as shown by the presence of mycelium and fruiting bodies, and, in some tests, infection of the germinated seedlings as demonstrated by symptoms on the young plants. Standard detection methods vary depending on which fungus is being tested. For example, in Phaseolus beans, 400 seeds per sample are placed between water-soaked sheets of paper towelling and incubated for 7 days at 20°C,

dark spots on the cotyledons are symptomatic of *Colletotrichum lindemuthianum* infection. In other tests the germination of seeds is deliberately suppressed to allow seedborne infection to develop. Thus *Brassica* seeds (1000 per sample) are placed on blotters irrigated with the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) solution and incubated for 11 days at 20°C in alternating cycles of light and dark to allow the pycnidia of seedborne *Phoma lingam* to develop. The germination of seeds is inhibited by 2,4-D solutions, allowing greater numbers of seeds to be tested. Similarly, carrot seeds (400 per sample) are tested for *Alternaria dauci* and *A. radicina* on triple-layer blotters soaked in sterile distilled water by incubating them for 3 days at 20°C, then at -20°C overnight followed by 7 days at 20°C in alternating cycles of light and dark for production of conidia.
Sterile media including sand, artificial composts, etc. can be used for the detection of certain pathogens. Results are based on the presence of symptoms typical of the organisms.

Staining and examination of the embryo
Staining methods are used for seedborne pathogens which cannot be detected by direct inspection or incubation methods. The standard method used in seed health testing is that of staining barley embryos of for the presence of loose smut (*Ustilago segetum* var. *tritici*) mycelium. The embryos of barley seeds (2000-4000 seeds per sample) are extracted over 24 h at 20°C in a 5% solution of sodium hydroxide. After clearing in lactophenol, the embryos are examined under magnification for the presence of the golden brown mycelium of the fungus in the scutellum of seeds. In certain laboratories lactic acid is preferred to lactophenol as a clearing agent.

Immunoassays
Serological tests for plant pathogenic bacteria have been known for nearly a century. The methods are based on the immunological principle that foreign molecules injected into the bloodstream of mammals stimulate the immune system of those mammals to produce specific antibodies which will recognize and bind to the antigens. Such antibodies recognize many chemical sites, referred to as epitopes, on target antigens; these are polyclonal antibodies. Immunoassays utilizing antisera produced against purified pathogens or extracts of pathogens have been effective in the detection of viruses but have had a more limited value for the detection of bacteria and particularly fungi.

The development of monoclonal antibodies (MAbs) has greatly increased specificity. Monoclonal antibodies recognize one chemical site (epitope) on target antigens and in that respect are homogenous and free from the variability common to polyclonal antisera. They can be selected to act at generic, specific, pathovar or strain levels.

The ELISA technique, has had considerable impact on the diagnosis of virus and bacterial diseases of vegetatively produced crops with a lesser but nonetheless important role in the diagnosis of seedborne diseases. There are many different forms of ELISA. Three of the main types are the direct (double antibody sandwich (DAS-ELISA)) method, the indirect method and the competitive assay. The direct method is most often used in seed health assays. Using the direct method, seed or seedling extract (i.e. the antigen) is selectively trapped and immobilized by solid-phase-specific antibody in microtitre wells. Enzyme-labelled antibody is then reacted with the immobilized antigen and, after removing unreacted enzyme-labelled antibody, the retained enzyme is assayed by adding a suitable substrate. Qualitative assays may be made by eye and visualization may he improved by the addition of substrates that give coloured hydrolysates. Quantitative assays are made using colorimetric or spectrophotometric equipment.

Molecular (PCR) Assays
The application of molecular nucleic acid tests in practical methods for the detection of seedborne organisms has not been fully exploited. The versatile nature of molecular methods has resulted in their use to supplement or replace established morphological, biochemical and serological techniques. Their introduction has been favoured in part, by their relatively quick development and validation time as opposed to serological and biochemical assays that take longer to implement. Once developed and validated, molecular assays can provide simple, quick, high-throughput diagnosis that is relatively cheap and easy to transfer to other laboratories. Initially, non-amplification assays were
used, but the need for pure cultures and poor repeatability did not make them suitable for routine diagnosis. Development of 'in-vitro' amplification by PCR is leading to specific, sensitive, robust assays for both pure cultures and, more importantly, for the detection and identification of organisms directly from seed and plant material.
Nucleic acid-based methods tend to be relatively expensive to apply and the advantages of these more rapid methods have to be considered against the lesser cost, but greater inconvenience to the grower, of the longer period required achieve identification of a pathogen using isolation-based methods. At present, a major disadvantage of nucleic acid methods in seed health testing is that of quantification. The technology is available and under evaluation, however, although the technology is relatively simple to use, the cost of the necessary equipment remains prohibitive for use in routine seed health testing.

Meeting future challenges

It is important that, as new and more advanced detection methods are introduced, their performance should be subjected to critical appraisal to establish the limits of their analytical and diagnostic sensitivity and specificity, ideally to demonstrate that they are at least as effective as the techniques, which they seek to replace.

The time taken to complete a diagnostic test can be critical for the release of seed. It is necessary to determine the number and frequency of tests that can be completed within a limited time span, a constraint introduced in certain circumstances by the need to sow the seed bulk within a matter of weeks. The development of rapid and more advanced methods for the detection of seedborne organisms will increase the frequency of some diagnostic tests and in that respect will benefit commercial seed testing. It is important that, as new and more advanced detection methods are introduced, their performance should be subjected to critical appraisal to establish the limits of their analytical and diagnostic sensitivity and specificity, ideally to demonstrate that they are at least as effective as the techniques, which they seek to replace. The ISTA Plant Disease Committee has recently implemented a programme of method validation. This method validation programme is open, any method developer or test kit manufacturer may submit a method for validation. Details of the programme can be obtained from the ISTA Secretariat or the ISTA online Website.

Industry Initiatives in Seed Health:

The Example of Vegetables

By Radha Ranganathan, International Seed Federation
Ruud Scheffer, Chairman ISHI-Veg Technical Co-ordination Group and
Harrie Koenraadt, Naktuinbouw, The Netherlands

The twofold responsibility the seed industry assumes in the area of seed health: to deliver good quality and healthy seed to farmers and seed producers, and to respect international phytosanitary regula-

Radha Ranganathan

Ruud Scheffer

Harrie Koenraadt

Introduction

The International Seed Federation (ISF) represents the mainstream of seed trade and the plant breeding community in the world. To facilitate the free movement of quality seed through fair and reasonable regulations is one among its many goals. And therein evolves the twofold responsibility the seed industry assumes in the area of seed health: to deliver good quality and healthy seed to farmers and seed producers, and to respect international phytosanitary regulations.

ISF members fund the International Seed Health Initiative (ISHI), which through its exclusive focus on seed health is perhaps unique in the world. Under the umbrella of ISHI, seed companies exchange information on seed-borne diseases and jointly develop methods for their control. The International Seed Health Initiative for Vegetable Crops (ISHI-Veg) was chartered in 1994 by the vegetable seed industries in the Netherlands and France and was soon joined by the vegetable seed industry in the United States, Israel and Japan. These countries together represent the production of over 75% of the world's vegetable seed supply. Public sector institutions Naktuinbouw and Plant Research International (NL), Volcani Centre (IL), Groupe d'Étude et de contrôle des Variétés et des Semences (GEVES) (FR), the Horticultural Research Institute (UK) and the Canadian Food Inspection Agency (CFIA), and private laboratories such as STA Laboratories Inc. (US) collaborate with ISHI-Veg member companies adding significant value to the work done.

Working together as an international group of more than 40 seed pathologists ISHI-Veg assesses, develops and disseminates information on test protocols for economically important seed-borne pathogens of vegetable crops. The combination of researchers and practitioners presents ISHI-Veg with the opportunity to develop protocols using both well-tested and novel approaches. This paper describes the practical and innovative activities of ISHI-Veg in securing the delivery of healthy seed.

ISHI-Veg Accepted Methods

Seed health testing is an integral tool for all vegetable seed companies in seed-borne disease risk management and is a part of the entire disease control program. The methods used are techniques either generated internally through a developmental process or those available for public use.

For a method to be accepted by ISHI and be considered as suitable for further development as a reference method, it must be either described and available for public use or published in a peer-reviewed journal, and meet the approval of the technical group within ISHI-Veg responsible for the relevant vegetable commodity. The aim when developing a reference method is that it best meets the needs of the laboratory conducting the test, often the seed producer, and its clients.

Most methods then go through a validation process that includes extensive comparative testing of the protocol. The process consists of checking the parameters of the protocol - sensitivity (of the assay in terms of percent-infected seed or target pathogen quantification, for instance), specificity (e.g. in identifying a range of isolates of the pathogen from different geographical regions and race), repeatability and reliability. Historical use, peer review, research in public institutions, and unbiased comparative testing by other groups also contribute to accomplishing the task.

Comparative Testing by ISHI-Veg

Seed health tests differ from country to country and the results from one country may not be accepted in another. A measure of ISHI-Veg's success lies in the international acceptance of its seed health tests. Comparative tests in many laboratories and countries are essential components of ISHI-Veg's activities. And to ensure reliable results the selection of participating labs that are familiar with the tests is critical.

Comparative tests require the availability of naturally infected seed lots. For some crop/pathogen combinations there is a lack of naturally infected or contaminated seed with the result that comparative tests are delayed. In such cases ISHI-Veg examines the use of artificial reference materials or has artificially infected seed lots.

Artificial Reference Materials

Bacterial preparations containing a specified quantity of viable bacterial cells were developed and used as reference material at Plant Research International. Preservation was optimised by freeze-drying bacteria (*Clavibacter michiganensis* subsp. *michiganensis* (Cmm), *Pseudomonas savastanoi* pv. *phaseolicola* (Psp), *Xanthomonas axonopodis* pv. *phaseoli* (Xap) and *Xanthomonas campestris* pv. *campestris* (Xcc) among others) in paper discs. The advantages of this approach are that the viability in time, the genotype, and the number of colony forming units of the target pathogen are well known or predictable. In addition, provided these reference materials are commercially available at a reasonable price, an unlimited supply of reference material can easily be made available.

A disadvantage of the use of such reference materials is that the 'real world' of seed testing cannot be simulated. For instance, the extraction kinetics of target pathogens from naturally contaminated seed lots crucial for detection of Cmm from treated tomato seed lots and Xcc from cabbage cannot be evaluated. Also the wide genetic variability of Cmm, as demonstrated by Giora Kritzmann of the Volcani Center (pers. comm.), shows that such reference materials cannot be used to answer all questions. Neither can a 'standard' laboratory execute the production of such bacterial reference materials, as special equipment is needed.

Artificially Contaminated Seed Lots

Inoculation of plants by a target pathogen with the aim of obtaining artificially contaminated seeds has not yet been widely exploited. Biggerstaff et al. (2000) successfully produced Cmm-infected seeds through inoculation of tomato plants and ISHI-Veg is trying to produce tomato seed lots infected with Cmm. It is planned to produce watermelon seeds infected with *Acidovorax avenae* subsp. *citrulli* (bacterial fruit blotch), an important crop/pathogen combination for the seed industry.

'Dilution' of Naturally Contaminated Seed with Healthy Seed and the Use of Preground Flour

A not altogether rare practice is one where comparative tests are made to evaluate the performance of participating laboratories. In the past equal numbers of sub samples from one heavily contaminated sample, two or three lightly contaminated samples, and one healthy sample were tested. The results of such tests were difficult to evaluate, especially when lightly contaminated samples were used, due to variation in the number of infected seeds per sample/participant. Using a relatively limited number of lightly contaminated sub samples it was difficult, if not impossible, to conclude whether laboratories missed infected samples or whether they received samples that had fewer infected sub samples.

An alternative approach is currently under investigation in Naktuinbouw. Several large seed lots infested by Lettuce Mosaic Potyvirus (LMV) stored at Naktuinbouw were used for a pilot. From four heavily contaminated seed lots, large pools of 10-fold dilutions (1x, 10x, and 100x) were prepared using one large healthy seed lot. From each dilution of every seed lot 20 sub samples were prepared. All the sub samples were renumbered to obtain a completely blind comparative test. In the pilot the results were as expected. In all sub samples of the 1x dilution LMV was detected. Depending on the infection rate of each seed lot, the number of infected sub samples decreased to zero in the 10x or 100x dilution. Even more information could be obtained when fewer sub samples from the undiluted positive samples and more sub samples from the 10-fold dilutions were used.

The flour of pea and squash seeds infected with Pea Seed-borne Mosaic Potyvirus (PSbMV), Pea Early Browning Tobravirus (PEBV) and Squash Mosaic Comovirus (SqMV), respectively, have also been used in comparative tests. Seeds were ground by the test-organiser to fine flour and distributed to participating laboratories to ensure that all had identical sub samples of flour. Data from these comparative tests revealed that almost all participants detected the virus in the sub samples. A big advantage of this approach is that since all participants receive identical sub samples, outliers can be easily identified. A limitation of this approach, however, is that it is best suited for large seeds that can be easily ground. In addition the virus must be stable and distributed evenly in the flour.

Although these formats of comparative testing are laborious for the organiser, especially when many laboratories participate, it is easy to identify a method or a laboratory with many false positive or false negative sub samples.

The Use of Multiple Methods

The ISHI experience has shown that using one method or a common methodology in all testing facilities is not always feasible; on the contrary ensuring consistent results from all testing facilities is the most important factor in seed health testing. Multiple methods where the results obtained from two or more methods are compared with that obtained from using only the accredited or standard method may provide an alternative.

Multiple methods have become accepted in clinical laboratories where there are good reasons to deviate from an existing standard method. For instance, labs may have expertise that allows for the implementation of another method or a very high or low numbers of samples, which for reasons of economy demand an alternative to the standard, or they may want a method that is cheaper, more sensitive or specific. Any of these reasons may result in the implementation of an alternative to the standard method.

Clinical laboratories have one major advantage over vegetable seed companies and the test laboratories related to them: there are many more clinical labs and reference materials are more easily available. In the Netherlands for instance, the monitoring organization SKZL organizes weekly reference tests at all participating laboratories. This allows SKZL to observe even gradual deviations of a specific test carried out by a lab, deviations such as a loss of sensitivity attributable to a spectrophotometer requiring maintenance.

In the field of medicine multiple methods have been shown to generate identical results in comparative tests where the performance of each laboratory and their method is evaluated rather than just the method. In the case of seed, the 'dilution method' described in the previous section was used to compare the performance of laboratories and not methods. If the methods used by the participating laboratories are not identical, it adds another important factor to the equation. Methods must be first investigated carefully in one or a few laboratories using a larger number of samples with different infections rates, more variation in genotypes, etc. If more than one method is used in a comparative test, the inevitable noise of inter-laboratory variance will most likely confound the results checking for equivalence of methods. This phenomenon has been seen in most, if not all, comparative tests in which the 'in-house and well known' method was compared with 'new and therefore unknown' methods. The authors believe that the comparison of different methods through elaborate com-

parative testing in several laboratories without prior and ample basic research at one location would not be very useful.

Despite the limited number of vegetable seed testing laboratories and the laborious task of first comparing methods before the performance of labs, there is good reason not to reject the idea of multiple tests. Relying exclusively on an older but 'standard' test could prove risky particularly in view of the rapid developments in technology. If a seed company had say, a quick and sensitive DNA chip test in routine use and if in a series of reference tests it was demonstrated that this method was superior or equivalent to the standard it should be accepted as an alternative to the standard.

In practice multiple methods are used today and differences between the protocols are minor. Examples are the use of different selective media, or differences in the extraction of bacteria from seeds, like grinding, shaking the seeds in a medium, or using a stomacher. Multiple methods based on radically different technologies like immuno-fluorescence vs. a DNA-based test are still unusual. An exception is the now rarely used ISTA method in which Lettuce Mosaic Potyvirus could be detected through either a grow-out of lettuce seeds or through a bioassay on *Chenopodium* plants (1981).

As part of its Performance Based Approach adopted during the Extraordinary Meeting in Bolivia in July 2002, ISTA also plans to use multiple methods to test for the presence of genetically modified organisms in seed.

Accepting multiple methods still stresses the need for generally accepted standard methods. Such reference methods are the 'standards' to which alternative test methods are compared, and used to 'calibrate' reference materials.

The ISHI-Veg Methods Manual

The ISHI-Veg manual lists methods accepted by ISHI-Veg and serves as a focal point and guide for the development of ISHI-Veg reference methods: methods that consistently provide reliable and repeatable detection of a specified seed-borne pathogen at a predetermined level of infection. The manual is available to all labs on the ISF website (www.worldseed.org).

For the purpose of this manual, status ratings for ISHI seed health testing methods are defined in the following manner.

o Category 1 - **An ISHI Reference Method**. The method has been through the ISHI comparative testing process, has been peer reviewed and accepted by ISHI.

o Category 2 - **An ISHI Accepted Method under Review**. The method has been published and/or is publicly available, and is commonly used in the seed industry for determining seed health. It is undergoing comparative testing or certain aspects of the test are being tested for retention or deletion from the method.

o Category 3 - **An ISHI Accepted Method**. The method has been published and/or is publicly available, and is commonly used in the seed industry for determining seed health. It is not undergoing a comparative test at this time.

When a method has been validated by ISTA and/or any other body this is also indicated.

Conclusion

In the foregoing sections we have illustrated how through international co-operation ISHI-Veg continually strives for reliable state-of-the-art test methods. Through this practical goal ISHI-Veg realises its mission - the delivery of healthy seed to customers on a worldwide basis.

The development of methods to test for seed health is complex. The epidemiology of diseases, different levels of infection in the seed, differences between climatic zones and the effect of such zones on infection rates are some variables that must be considered in developing reliable test methods, and particularly so in the field of bacterial diseases. Comprehensive studies that elucidate on all these aspects are often not possible to find, as data are usually scattered and inconclusive. The combined experience, data and expertise within ISHI-Veg allows for test methods to be developed that take into account the above-mentioned factors.

Clearly, scientific developments, changes in cultural practices of both seed companies and growers and changing customer needs add to the complexity. The comparative test program of ISHI-Veg and its aim to conclude on generally accepted standard methods are key efforts to make progress towards its mission. The careful evaluation of alternative test protocols does result in multiple methods being recognized and accepted. However, using ISHI's reference methods and the methods designated 'generally accepted', companies are able to make risk analyses specific to the conditions under which they operate, taking also other factors such as resistance in their varieties, seed production region, and the region where the seeds will be sold into consideration, all again to secure the delivery of healthy seed.

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R. Ranganathan, International Seed Federation, 7 Chemin du Reposoir, 1260 Nyon, Switzerland; Ruud Scheffer, Chairman ISHI-Veg Technical Co-ordination Group, 7 Chemin du Reposoir, 1260 Nyon, Switzerland. Harrie Koenraadt, Naktuinbouw, P O Box 40, 2370 AA Roelofarendsveen, The Netherlands.

Moisture Content Proficiency Test

By Ronald Don, ISTA Moisture Committee Vice-chair

How to volunteer to become leader of an ISTA Proficiency Test

It was the summer of 2002. It had been a long week: first there had been the 28 hour journey to Santa Cruz and that had been followed by 3 long days of Executive Committee Meetings. Sunday was my day off. Relaxing at the side of the pool of the Los Tajibos Hotel and Conference Centre after a 14 hour tour to the world heritage site at Samaipata - I was at my most vulnerable.

"Lovely evening Ronnie" was the greeting in unison from Harry Nijënstein (ISTA Moisture Committee Chairman) and Martina Rösch (ISTA Secretariat, Head of Accreditation).
I had to agree, it was.

"Wouldn't it be a good idea to have Moisture Content testing included in the next round of proficiency tests?" asked Harry.
Again I had to agree; moisture content testing is one of the original seed quality tests but moisture content has never been included in the proficiency testing program.

"Why are moisture content tests not part of the program?" Martina enquired.
I tried to explain as simply as I could - Unlike purity and germination testing where results are based on a qualitative characteristic of seeds like pure seed versus not pure seed or normal seedling versus not normal seedling, moisture content is a quantitative trait which is expressed as a percentage. Because of this the usual method of statistical evaluation based on Tattersfield can not be applied and this has resulted in the omission of moisture content testing from the normal program of proficiency tests. In 1998 there had been a pilot test using the species *Phalaris aquatica* to ascertain the most appropriate means of statistical evaluation and to determine the feasibility of including moisture content determinations future proficiency testing programs. The pilot went well and Dr Michael Kruse had developed a statistical evaluation procedure based on the general principles of Tattersfield. Michael had also tested the evaluation procedure on a moisture content comparative test that I had organised in 1999 and again it had been successful in identifying laboratories that may have had analytical problems with moisture content testing.

"Mmm" was the rely from Martina, "So there is no reason for moisture content to be excluded from future testing programs?"
Again I had to agree.

"What we need, Martina," said Harry "is someone with Ronnie's experience in preparing moisture content comparative tests to volunteer".
Again I had to agree. I even stated that I would be glad to volunteer but that the majority of seed production in Scotland involves cereals and that the first moisture proficiency test should be on a small seeded species that didn't need to be ground. This I thought was the perfect justification/excuse for not taking on this piece of ISTA work.

"That's not a problem", said Martina, "we have arranged for Rita Zecchinelli, from the Proficiency Test Committee, to send you samples of the clover seed that will be used for the Germination, Purity, and Other Seed Determination Proficiency test that will be sent out in February 2003."

But what about phytosanitary requirements?,….timing?,…..procedures….? Harry and Martina had answers to all my questions and there was no way I could wriggle out of it; I was the volunteer who would prepare the ISTA Moisture content proficiency test even although I can't quite remember actually volunteering. **It was a stitch-up!**

The Samples and Preparation Instructions

The samples arrived from Rita Zecchinelli in November - 3 bags of about 5 kg each of the same lots that would be used for the other parts of the clover proficiency test. We would have plenty time to prepare the samples and send them to ISTA for distribution by the end of February, or so it seemed! Around about the same time Martina was kind enough to send me the Protocol to be used for by Proficiency test leaders: *"REFEREE SAMPLES PREPARATION INSTRUCTIONS FOR REFEREE TEST LEADERS - PURITY, GERMINATION AND OTHER SEED DETERMINATION TESTS."* These guidelines had be adopted by the Proficiency (Referee) Committee to ensure the ISTA Proficiency Program is incorporating the basic principles for proficiency testing programs outlined in ISO Guide 43. In particular they ensure that proficiency samples, sent to participating laboratories, are homogeneous.

The instructions, twelve pages in all, were very detailed and the procedures looked much more complex than the ones I had used to prepare cereal samples for the moisture content comparative test involving 12 laboratories. In addition there was no mention of preparing samples for a moisture proficiency test. Martina told me

The sculptured rock at Samaipata bears outstanding witness to the existence in this Andean region of a culture with highly developed religious traditions, illustrated dramatically in the form of immense rock sculptures.

The pool at the centre of the Los Tajibos hotel and conference complex in Santa Cruz, Bolivia where the first Extraordinary Meeting of ISTA was held in July 2002.

not to worry and just adapt the procedures given for purity, germination and other seed determination tests.

Adjusting Sample Moisture Contents

This was the easy bit.

For the proficiency test it was decided that the samples sent to participating laboratories should have a range of different moisture contents. To achieve this objective the three lots of clover seed were subjected to the following treatments:

- Lot A - sent to laboratories without any moisture content adjustment.
- Lot B - placed in an oven at 30°C for 6 weeks to reduce moisture content.
- Lot C - placed in a room at a temperature of 20°C and 95% Relative humidity for 7 days to increase moisture content.

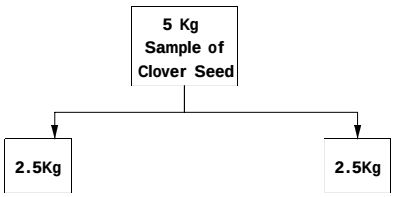
Preparation of Proficiency Samples

This was the difficult bit!!!

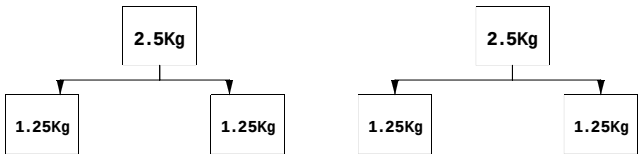
Monday, Tuesday and Wednesday

To prepare the 160 samples, required by the Secretariat, from each lot was complex and time consuming. The laboratory was busy and the job of preparing the samples was allocated to me with help from Margaret Orr, who normally provides the laboratory with administrative support. Mixing and dividing was achieved using a Centrifugal Divider and each set of samples took a full day to prepare in a process that involved 10 steps:

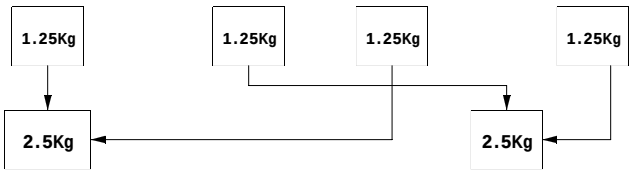
Step 1
Sample is mixed three times and then divided into two.



Step 2
Each half is mixed three times and then divided into two halves giving 4 samples halves.

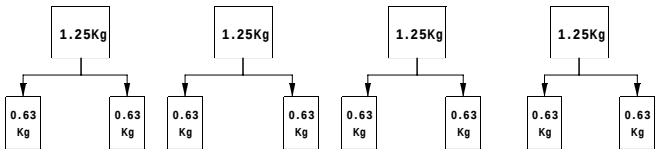


Step 3
Alternate quarters are combined to give two half samples.

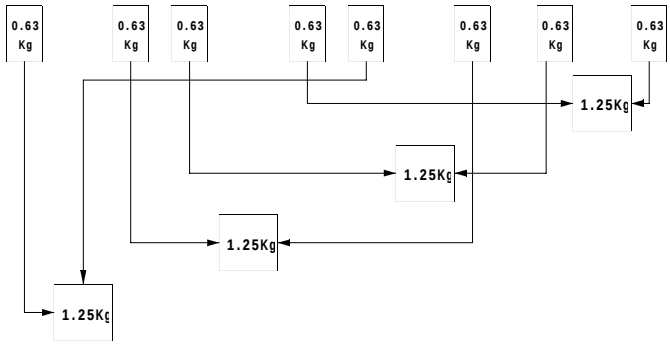


Step 4
This is a repeat of Step 2 - each half is mixed three times and then divided into two halves giving 4 samples.

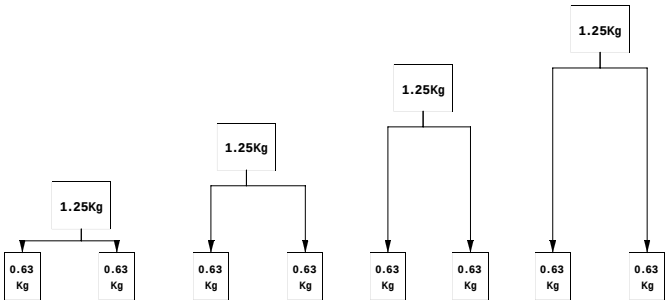
Step 5
The four quarters are each mixed three times and then divided into two halves giving 8 samples.



Step 6
The eighths are recombined to give 4 samples.

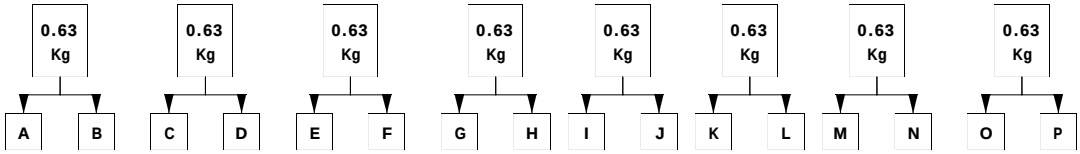


Step 7
A repeat of Step 5 - The four quarters are each mixed three times and then divided into two halves giving 8 samples.



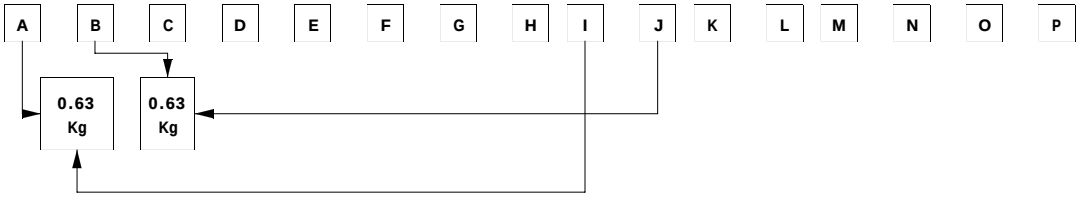
Step 8
The eight eighths are each mixed three times and then divided into two halves giving 16 samples.

Step 9

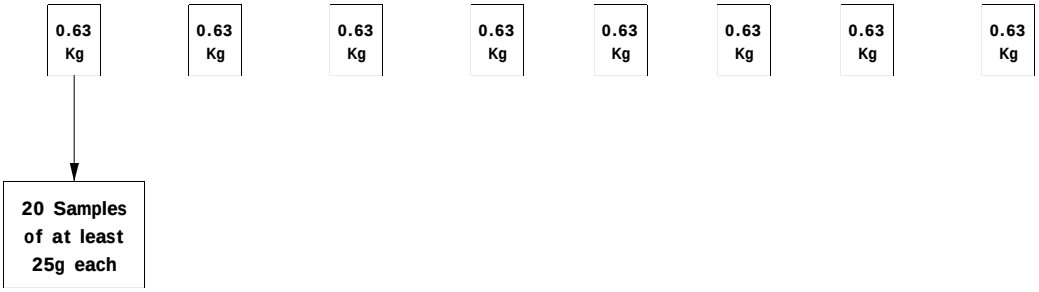


The sixteenths are recombined to give 8 samples.

Step 10



From each of the eighths 20 samples of at least 25 g are obtained using the spoon method of sampling. Each of these samples is placed in a foil bag, which is heat sealed and labelled according to instructions from the Secretariat.



This may seem simple when written down like this but believe me it was a major operation involving almost every mixing bowl in the seed laboratory. In addition, all seed not in the process of being mixed and divided had to be placed in moisture proof containers to prevent any changes to their moisture content that would have resulted in differences in moisture content between samples of the same seed lot.

Each lot took 10 hours to divide down into the 160 samples required for the proficiency test. During the period of preparation daily contact was maintained with Gerhard in the ISTA Secretariat. Gerhard would be responsible for the dispatch of the samples to ISTA Laboratories and he also gave Margaret and I endless encouragement. We had started the preparation on Monday morning and at 18:00 on Wednesday the last sample (sample 480) had been sealed in its foil bag and labeled. I was exhausted and needed a day off. I E-mailed Gerhard to say we had finished and left Margaret instructions to pack the samples and send by courier to Gerhard.

Thursday

Whilst I was enjoying my day off, Gerhard E-Mailed Margaret to ask for the results of tests to prove that the samples we had prepared were homogenous. This was something I had forgotten about. Margaret had to arrange for 10 samples from each lot, randomly chosen by ISTA, to be tested for moisture content. I must have been popular, and it's a wonder my ears were not ringing. But the staff in the lab are very obliging. Thirty moisture content tests were completed by 15:00, and the results E-mailed to ISTA.

Friday

I was informed of the previous days developments and told that Annabella was due a box of chocolates for doing all the moisture content tests at such short notice. Looking at the results of the tests I thought it was Margaret and I that were due a box of chocolates - the 10 results from each lot were within ±0.2% of each other. I was confident that H-test analysis by Günter Müller, Vice Chairman of the Proficiency Test Committee, would show that the samples were fit for purpose and could be used for the Moisture Content Proficiency test. I was correct, Günter confirmed this within the hour and we could send the samples off to ISTA.

Margaret found a box big enough to accommodate all the samples. She carefully packed them by seed lot and sample number to make the job of the Secretariat easier when dispatching samples to participating laboratories. We had used just over 9 kg of seed but the weight of the foil bags and packing meant that the final parcel had a weight of 17 kg - too heavy for airmail! It was sent by courier - cost £112.

Dispatch of Proficiency Samples to ISTA Laboratories

The samples were dispatched from the ISTA Secretariat Office.The photograph shows ISTA Secretariat Staff hard at

Two weeks later

ISTA clover proficiency samples arrive in the laboratory for testing. Usually they wouldn't be given any special thought. But these samples were special -we will never look at proficiency/referee samples in the same way again. We now appreciate the work and effort required in providing laboratories with these samples.

Temperature Measurement and Control in ISTA Laboratories

Guidelines for Laboratories and Auditors

By Ronald Don, ISTA Moisture Committee Vice-chair

Temperature

Temperature is one of the most commonly measured physical quantities but its basis is not widely understood. Whereas the units of other quantities, such as mass and time are based on real physical entities temperature is founded on a theoretical set of conditions. So whilst the perfect kilogram is in Paris and time is based on atomic transitions in a caesium atom, temperature is based on the thermodynamics of perfect systems, such as ideal gases. This results in the thermodynamic temperature scale measured in Kelvins (K), which is unattainable. To overcome this we do the next best thing and use imperfect thermodynamic systems to achieve a working temperature scale as near to the theoretical one as we can get.

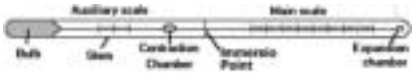
Temperature is an important factor in a number of tests carried out by ISTA Laboratories. It can affect the outcome of Germination, Moisture Content, Tetrazolium and Vigour tests and laboratories must take measures to control and monitor temperature. This set of guidelines has been drawn up to assist laboratories and auditors when they are considering the arrangement for temperature measurement.

Temperature Measurement

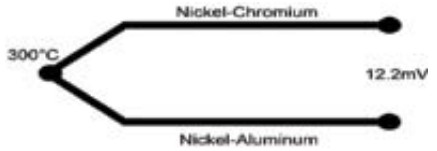
The two most common methods of measuring temperature are using thermometers or thermocouples

THERMOMETERS

A thermometer is an instrument that measures the temperature of a system in a quantitative way. The most direct 'regular' way is a linear one. For example, the element mercury is liquid in the temperature range of -38.9° C to 356.7° C. As a liquid, mercury expands as it gets warmer; its expansion rate is linear and can be accurately calibrated.



The mercury-in-glass thermometer contains a bulb filled with mercury that is allowed to expand into a capillary. Its rate of expansion is calibrated on the glass scale.



When wires of different metals are fused at one end and heated, a current flows from one to the other. The electromotive force generated can be quantitatively related to the temperature and hence, the system can be used as a thermometer - known as a thermocouple. The thermocouple is used in many electronic/digital thermometers and many different metals are used, for example - platinum and platinum/rhodium; nickel- chromium and nickel-aluminum.

Temperature Measurement in ISTA Laboratories

A variety of different equipment is used to measure temperature in ISTA laboratories. Some use thermometers / temperature probes with accuracy of ± 0.1°C whereas others use thermometers where it would be difficult to achieve a temperature reading

with an accuracy of ± 2°C.

Recommendation for Temperature Measurement in ISTA Laboratories

- Laboratories should use temperature measurement instruments can be read accurately to at least 0.5°C and conform to ISO 386.
- For Accelerated Ageing Vigour Tests the

instrument must be read accurately to at least 0.1°C.

Calibration and the ISTA Standard

Appropriate calibration samples, reference materials and reference standards of measurement shall be held by the laboratory, and should be used for calibration and reference purposes only. Examples include calibration samples for seed blowers, standard buffer solutions for pH meters, calibration weights for balances, seed reference collections for the identification of unknown seed.

Calibration of temperature

Some laboratories have all probes calibrated externally, others use externally calibrated probes to calibrate their working probes, others calibrate their thermometers with steam and ice and others do no calibration what so ever.

Calibration of Thermometers - External Calibration

RECOMMENDATIONS

The current working temperature scale is the International Temperature Scale of 1990 (ITS-90) and is measured in degrees Celsius (°C).

- When a thermometer is calibrated externally it should be to IPTS-90 or to an agreed previous temperature scale such as IPTS-68 or even IPTS-48.
- External calibration should be repeated every 5 years and this should be supplemented by annual in-house ice point calibration.
- Any probe whose temperature reading differs from the standard by more or less than ±0.5°C should be removed from service.
- For Accelerated Ageing Vigour Tests the limit is ±0.1°C

Calibration of Thermometers - Against an External Standard

RECOMMENDATIONS

- When Calibrating a batch of thermometers against an externally calibrated probe the calibration should be carried at temperatures within the normal working range and

in conditions where fluctuation in temperature between readings is limited.

- Where working probes are calibrated against an externally calibrated probe this should take place at least once a year.

- Any probe whose temperature reading differs from the standard by more or less than ±0.5°C should be removed from service (For Accelerated Ageing Vigour Tests the limit is ±0.1°C)

Calibration of Thermometers - International Ice Point Method

The ice point may be realised in an insulated flask or vessel containing an ice-water melting mixture. The ice particles should be no more than a few millimetres in diameter and the water and ice should be pure or prepared from de-ionised water, which is air saturated.

For high precision the thermometer should be maintained in the mixture for 10 minutes prior to reading. In theory accuracies of ±0.001°C may be achieved but in practice ±0.005°C is more likely.

RECOMMENDATIONS

- International Methodology should be followed.

- Calibration should take place at least every year.

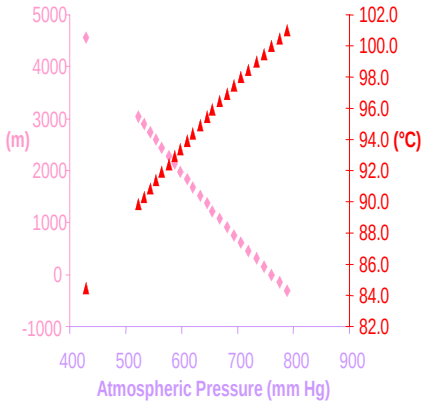
- Any probe whose temperature reading differs from the ice point by more or less than ±0.5°C should be removed from service.[1]

Calibration of Thermometers - Steam/Boiling Water Method

The boiling point of water varies with atmospheric pressure and this is dependent on both weather conditions and altitude. At sea level with an atmospheric pressure of 760mm Hg the boiling point may be 100°C but in Kathmandu in Nepal at an altitude of 1310m and atmospheric pressure of 650mm Hg the boiling point is 95.5°C.

RECOMMENDATION

- Calibration of temperature probes using steam/boiling water should not be used by ISTA Laboratories



Calibration of Thermocouples, Electronic Meters and Temperature Data Loggers

RECOMMENDATIONS

- The procedures for the calibration of thermocouples and temperature probes of electronic meters and data loggers should be the same as that adopted for thermometers. In other words they can be calibrated externally, or in-house

· against an externally calibrated probe or thermometer; or

· using the ice point method.

- As with thermometers, any probe that differs by more than ±0.5°C from the standard should be removed from service (±0.1°C in the case of Accelerated Aging Vigour tests).

Records of Calibration Checks

RECOMMENDATIONS

- Calibration Checks should be recorded and be available for inspection by auditors. The easiest way to do this is in a Thermometer/Probe Register.

- Records should be archived for a period of at least 5 years.

- Adjustments should be made for differences between probe temperature and that of the calibrated standard. For example if a probe reads 19.6°C when the calibrated thermometer/probe reads 20°C, and the probe is to be used to monitor a germination room which has a performance requirement of 20±2°C the limits using the probe will be 17.6 - 21.6°C.

Total immersion and partial immersion thermometers

Total immersion thermometers are designed to be totally submerged in the media whose temperature is being measured. They are distinguished by the engraved suffix '/ TOTAL' or '/ TOT IMM'.

Partial immersion thermometers should only be immersed to the indicated depth. They have a suffix that indicates the immersion depth, e.g. '/ 100 MM IMM' should be immersed to the depth of 10 cm. They may also have an engraved ring on the stem indicating the immersion depth.

Measurement of Temperature - Total immersion thermometers and thermocouples

RECOMMENDATIONS

- Total immersion thermometers and thermocouples can be used to monitor the temperature of incubators, walk in germinators, ovens and fridges.

- For ease of taking measurements and to reduce dramatic temperature fluctuations that can result from the opening of doors of apparatus thermometers and probes should be submerged in glycerol or sand.

Measurement of Temperature - Partial immersion thermometers

RECOMMENDATION

- Partial immersion thermometers can be used to monitor the temperature of incubators, ovens and fridges through an external aperture on the apparatus provided that they can be immersed to the required depth with-

Temperature Measurement and Control in ISTA Laboratories is continued on page 30.

Measurement of Temperature - Copenhagen Tanks

Total and Partial immersion thermometers can NOT be used to monitor the temperature of Copenhagen Tanks or Germinators scince it is not possible to meet immersion requirements.

Thermocouples with contact probes must be used.

in the apparatus.

Measurement of Temperature - Frequency

Many laboratories now use data-loggers or other temperature monitoring equipment that constantly monitors temperature and records of readings are available on an hourly basis or less. Such records should be archived for a period of at least 5 years and be available for the inspection of auditors who may want to ascertain the temperature of a particular piece of apparatus on a specific date.

RECOMMENDATIONS for MANUAL RECORDING

- For constant temperature equipment at least 3 readings must be recorded per day at regular pre-set times. If records show that the equipment is stable, in terms of temperature, with variations of less than 1°C between readings the recording frequency can be reduced to once per day. However, if there is any indication of a change in performance recording frequency must be increased.
- For alternating temperature equipment at least 3 readings must be recorded per day at pre-set times. The timing of these readings must be such that both temperature phases are monitored.
- Laboratories should plot readings from individual pieces of apparatus against time, as this is the easiest way to notice any changes in the performance.
- For alternating temperature equipment monthly checks are required on the change over time between high and low phases. This should be no more than 3 hours.
- Where records show that a piece of apparatus is stable then measures to take readings at weekends and public holidays are not required. Where apparatus if unstable measures should be taken to ensure readings are taken. This might involve the use of a max/ minimum thermometer of a small individual logger.

Measurement of Temperature - Number of Measuring Points

RECOMMENDATION

- For Germination /Temperature Rooms or Cabinets with an area of 20m2 or more there must at least three measuring points.

Measurement of Temperature - Temperature Profiles

RECOMMENDATIONS

- Before accepting a piece of temperature controlled equipment (ovens, incubators, fridges, Copenhagen tanks, germinators, propagators, germination rooms, etc.) into service a laboratory must ascertain the temperature profile of the equipment to ensure that it is fit for purpose.
- There should be a minimum of 9 check points covering different combinations of height, depth and breadth, as appropriate. For example, for incubators, fridges and ovens temperature should be recorded on top, middle and bottom shelves and on each shelve the temperature should be measured at 3 points - front (next door), middle and back.
- The temperatures of all points measured must be within ± 2°C[2] before a piece of equipment is accepted into service.
- Once in service profiles are only measured again if a piece of equipment undergoes repair or major service. ■

[1] For Accelerated Ageing Vigour Tests the limit is ±0.1°C

[2] The Ageing Chamber for Accelerated Ageing Vigour Tests must have a profile of less than ±0.3°C when operating at a temperature of 41°C

ANNOUNCEMENT

ISTA Handbook for Seedling Evaluation, 3rd Edition, 2003

By J. Bekendam, R. Schmitt-Grob and R. Don

ISBN 3-906549-26-7

This handbook is a most valuable guide for seed analysts the world over. Although the 'International Rules for Seed Testing' define normal and abnormal seedlings in considerable detail, the additional help provided by this handbook, with its detailed instructions and many illustrations, is vital if the principles of seedling evaluation are to be applied uniformly. The 3rd edition also includes many tropical, subtropical, flower and tree species.

What is new about the 'ISTA Handbook for Seedling Evaluation'?

The first section of the new addition is aimed mainly at the trainee seed analyst and trainers and contains background information on the elements of seed biology, an understanding of which is considered essential for seed analysts. A large number of new diagrams as well as the classic Dutch diagrams from the 2nd edition give life to the text and help explain the finer details of physiology and seedling evaluation. For the first time, general evaluation rules such as the 50% rule for the evaluation of cotyledons are considered in depth and explained with the use of diagrams and colour prints of seedlings. The evaluation of different seedling types is fully illustrated by colour prints of normal and abnormal seedlings. Diagrams and plates are also used to explain in detail the intricacies of the evaluation of species, such as *Zea* and *Phaseolus*, and defects, such as physiological necrosis and negative geotropism.

It is planned to regularly update the new edition of the Handbook and to facilitate this in a binder format with individual sections adopting a QA numbering system. Amendments and additions to the handbook can be easily made without the expense of reprinting the entire publication.

ISTA Crop and Weed Species Survey

By Ken Allison, ISTA Purity Committee Chair

There is a need for ISTA to have a "universal list" of crop and weed species that analysts in all ISTA-accredited laboratories should be able to identify. There are two main reasons for this list:

1. The Proficiency Test Committee and Test Leaders could use the species on this list when choosing crops and inclusions for referees.
2. Audit teams could expect all accredited laboratories to have the listed species, as a minimum requirement, in their reference collections.

In developing such a list, the ISTA Purity Committee felt that it was important to have input from a broad spectrum of ISTA members to ensure that the list is relevant to all regions and climate zones. It was especially important that the list not be based solely on familiar temperate crops and weeds. A survey form was sent out to ISTA members from the Secretariat asking them to rank their top 10 crop species and their top 25 weed species.

Results were received from 65 laboratories in 40 countries, one in Africa, two in the South Pacific, six in Asia, 25 in Europe, three in North America and three in South America. As you can see, the information we received is still heavily weighted in favour of the temperate zone crops. It would be valuable to have more data from ISTA laboratories in tropical and subtropical countries, so that these regions would be better represented. Please contact the ISTA Purity Committee Chairperson if you would like to contribute.

The data on kinds have been summarized. Table 1 is a list of the top 30 species of crops reported by respondents. The score is based on their ranking (1-10) by the laboratories and the number of samples received each year. Table 2 is a list of the top 50 species of weeds reported by respondents. The score is based on their ranking (1-25) by the laboratories. These are the short lists; the next step will be to decide how many of these species should be included in the final list. For example, there doesn't seem to be much point in including both species of Lolium and their hybrid, since they are so difficult to separate from each other. Again, any suggestions would be welcomed.

Thank you to all who contributed data for this important project and to Bettina Kahlert at the ISTA Secretariat who typed the numbers into a spreadsheet.

	Crop Species	Score	Labs
1	*Zea mays*	4558	33
2	*Triticum aestivum*	3541	47
3	*Hordeum vulgare*	2480	43
4	*Lolium perenne*	1431	25
5	*Glycine max*	879	17
6	*Secale cereale*	584	19
7	*Avena sativa*	458	31
8	*Festuca arundinacea*	243	9
9	*Pisum sativum*	234	25
10	*Medicago sativa*	151	20
11	*Festuca rubra*	105	12
12	*Triticum durum*	72	7
13	*Helianthus annuus*	70	17
14	*Lolium multiflorum*	58	15
15	*Phleum pratense*	38	16
16	*Beta vulgaris*	32	12
17	*Poa pratensis*	25	10
18	*Sorghum xdrummondii*	18	2
19	*Brachiaria brizantha*	16	1
20	*Trifolium repens*	14	9
21	*Brassica napus*	14	12
22	*Festuca pratensis*	13	11
23	*Panicum maximum*	12	3
24	*Sorghum bicolor*	12	13
25	*Trifolium pratense*	12	12
26	*Gossypium* spp.	10	5
27	*Allium cepa*	9	8
28	*Lycopersicon esculentum*	9	8
29	*Oryza sativa*	7	7
30	*Dactylis glomerata*	7	8

	Weed Species	Score	Labs
1	*Chenopodium album*	733	42
2	*Rumex crispus/obtusifolius*	610	41
3	*Polygonum aviculare*	457	28
4	*Elytrigia repens*	441	27
5	*Galium aparine*	399	23
6	*Echinochloa crus-galli*	373	20
7	*Amaranthus spp.*	332	23
8	*Bromus hordeaceus*	306	14
9	*Tripleurospermum perforatum*	296	12
10	*Stellaria media*	288	19
11	*Avena fatua*	281	18
12	*Viola sp.*	246	19
13	*Poa annua*	240	16
14	*Vulpia spp.*	228	10
15	*Apera spica-venti*	225	13
16	*Raphanus raphanistrum*	213	19
17	*Rumex acetosella*	207	17
18	*Lapsana communis*	179	15
19	*Sinapis arvensis*	179	13
20	*Alopecurus geniculatus*	174	14
21	*Capsella bursa-pastoris*	166	16
22	*Plantago lanceolata*	155	10
23	*Fallopia convolvulus*	150	25
24	*Convulvulus arvensis*	147	11
25	*Thaspi arvense*	136	14
26	*Setaria viridis*	120	7
27	*Galeopsis tetrahit*	119	8
28	*Solanum nigrum*	116	9
29	*Bromus sterilis*	113	7
30	*Centaurea cyanus*	96	7
31	*Polygonum persicaria*	96	5
32	*Cirsium arvense*	95	10
33	*Ipomoea* spp.	93	6
34	*Myosotis* sp.	84	15
35	*Digitaria sanguinalis*	81	7
36	*Alopecurus myosuroides*	76	7
37	*Panicum* spp.	76	5
38	*Cirsium vulgare*	72	8
39	*Poa trivialis*	71	5
40	*Dactylis glomarata*	68	5
41	*Geranium* sp.	68	9
42	*Silene* sp.	68	7
43	*Spergula arvensis*	68	7
44	*Vulpia bromoides*	67	4
45	*Sonchus asper*	64	7
46	*Erodium cicutarium*	60	6
47	*Ammi majus*	59	3
48	*Rapistrum rugosum*	59	6
49	*Sida* spp.	59	4
50	*Melilotus* spp.	56	4

Flower Seed Testing

Preparation Work of the ISTA Handbook on Flower Seed Testing

By Zita Ripka, ISTA Flower Seed Committee Chair

The main work of the ISTA Flower Seed Committee (FSC) since 1998 has been to prepare material for a flower seed testing handbook. It is a challenge since no similar handbook was issued before. We are well aware of the fact that flower seed testing is not a highlighted area in the seed testing world but it is an important matter for those who make their living from flower seed multiplication and trade. So hopefully the future handbook will be a very useful guide for laboratories testing flower seeds.

The handbook will consist of two main parts. First the general part with chapters Introduction, Apparatus, Reference -and perhaps Method Validation Programme- which contain important but general knowledge about seed testing and will not be repeated in the working sheets later. The second part contains the working sheets with a series of procedures and detailed descriptions, instruction and conditions of the most important laboratory seed tests of a flower species or genera.

The main parts of a work sheet are:

- Title page: gives the botanical data of the species -botanical and common name and family
- Seed description: text and coloured photos in natural size and enlarged
- 1000 seed weight: data for information
- Purity test: pure seed definition, sample size
- Germination test: method, seedling description, coloured photo of normal seedlings and of the most frequent types of abnormal seedlings
- Tetrazolium test: method description and colour drawings to help evaluation
- Seed-borne diseases: list of pathogens, diseases, distribution, reference, CMI and test method
- Literature: list concerning the species

In the beginning of the work 6 genera were picked as the first task. These are: *Calendula, Gaillardia* and *Tagetes* from the Asteraceae family, *Dianthus* from Caryophyllaceae family, *Impatiens* from Balsaminaceae family and *Viola* from Violaceae family. The work had a uniform pattern:

1. Determine if the present method is correct: questionnaires were circulated among FSC members by working group leaders and from the evaluation of these it can be told if a method is correct or there are some need to change the conditions of the germination test or modify the seedling evaluation

2. Detect points where changes are needed: by comparative tests it can be detected where exactly the changes are needed. A working group leader organises the comparative tests. In the work of working groups 5-7 FSC members participate and sometimes there are members from other ISTA committees also from laboratories where they often test the given species.

3. Proposal for changes in the present method: discussions among FSC working group members and also with other ISTA committees and when it is needed organisation of repeated comparative tests

4. Draft text of the work sheet: by the report of the working group leaders the FSC chair prepares a draft text and circulate it for validation within FSC and after within ISTA

5. Issue of handbook work sheet: the finally accepted version.

To get the right answers to the professional questions we work together with the ISTA Germination and Tetrazolium Committees to find accurate test methods for every species. We also try to harmonize the test methods of ISTA and AOSA during this work and AOSA members also take part in our comparative tests and their evaluation.

We started consultations with members of AOSA and SCST as well as experts from seed industry, which revealed that at present it is unlikely to fully harmonize the two methods because of the economic considerations of SCST. (It means mainly the difference in the evaluation of normal and abnormal seedlings -presence and number of primary and secondary roots and physiological necrosis.) Therefore at the present stage in the handbook we cite the ISTA methods and point out the AOSA Rules as a note for information to the analysts interested.

Experience of the first 6 work sheets:
The most complicated and time consuming tasks were: the seed description part, to find the most frequent types of abnormal seedlings, to prepare TZ test method and collect seed-borne diseases.
For *Calendula*, *Dianthus* and *Viola* spp. - there was no difficulty in the preparation of the work sheets.
Gaillardia spp. -there was no difficulty only TZ method was new so it had to be checked.
Tagetes spp. -there are some trouble with the evaluation of seedlings. The question is whether seedlings with defective primary root and well-developed secondary roots should be considered normal or abnormal.
Impatiens spp. - for germination of *Impatiens walleriana* the addition of light was suggested in the method. Based on the comparative tests of FSC the new seedling evaluation group of *Impatiens* should be A.2.1.1.2.

Since e-mailing is general today our work became much faster and more communicative than before but I feel that we could improve it by making InterNet conferences on the ISTA website. (I mention it because it is the sort of work, which demands rather personal discussions among several people. We can experience friendly but animated professional discussions during ISTA workshops. I miss this feeling via bilateral e-mails.)

All this work was started by Lea Mazor former chair of FSC and completed by FSC members who are always willing to make this extra work to contribute to the main goal of ISTA: uniformity in seed testing. Working group leaders were for *Impatiens walleriana* and *Gaillardia*: Lea Mazor, *Viola tricolor* and *Petunia*: Sylvie Ducournau, for *Tagetes*: Aleta Meyr, for *Calendula officinalis*: Marcia Taylor, for *Dianthus*: Zita Ripka and for *Cyclamen*: Rita Zecchinelli. FSC also owes a lot of thanks to other members of ISTA who

helped our work with their kind and helpful cooperation. They are mainly the members of ISTA Germination Committee, in TZ testing the LUFA staff, in seed-borne diseases Prof. Wen-Shi Wu, Jim Sheppard in website matters and Bettina Kahlert in everything general. Dr. Norma B. Deginani and Dr Raul Pozner, the Darwin Institute of Botany, Argentina gave their assistance in seed description.

In the future work the preliminary questionnaires will be the basis of the work sheets where the result shows no difficulty or wish to change the present method. Comparative tests will be carried out only for genera or species where some changes are needed and for new species. This way we hopefully can quicken up the preparation of working sheets for the next species and genera.

Questionnaire on *Cyclamen* sp.
for the ISTA Flower Seed Committee working group
Return it please before 15 October 2001

to: Rita Zecchinelli - Laboratorio ENSE - Via Emilia km 307 26838, Tavazzano (LO) Italy
e-mail: ense-tavazzano@ense.it
Fax: +39 0371 760812
Tel.: +39 0371 761919-760135

Name: .....
Laboratory:.....
Number of samples tested a year (max): 0-10 10-30 30-50 50-100 +100
Average of the last 5 years:.....
Are you a practised analyst in germination test of *Cyclamen* sp.: yes no

Please answer the following questions:
1/ Indicate the frequency of seedling abnormalities by your experience:

Type of abnormality	Frequency
I. Primary roots	
I.2. Stubby	
I.3. Retarded	
I.8. Spindly	
I.10. With negative geotropism	
I.11. Glassy	
I.12. Decayed as a result of primary infection	
I.13. Only secondary roots developed	
II. Hypocotyl, epycotyl, mesocotyl	
II.2. Not forming a tuber	
II.3. Deeply cracked or broken	
II.6. Constricted	
II.10. Spindly	
II.11. Glassy	
II.12. Decayed as a result of primary infection	
III. Cotyledon	
III.3. Broken or othetwise damaged	
III.8. Decayed as a result of primary infection	
V. Terminal bud	
V.1. Deformed	
V.3. Absent	
VII. Seedling as a whole	
VII.1. Deformed	
VII.2. Fractured	
VII.4. Two fused together	
VII.6. Yellow or white	
VII.7. Spindly	
VII.8. Glassy	
VII.9. Decayed as a result of primary infection	

O none, x seldom, xx seldom to regular, xxx regular, xxxx regular to often, xxxxx often

2/ Is it neccessary to revise the germination methods in ISTA Rules Table 5A Part 3? Please indicate your suggestions in the following table:

	Substrate	Temp(ºC)	First count (days)	Final count (days)	Breaking dormancy
ISTA Rules	TP;BP;S	20;15	14-21	35	KNO3-Soak in water 24 hours

Changes.....

3/ Do you think that seedling evaluation group A 2.1.4.3. is correct? Yes
No, I think .....would be more adequate, because
.....
.....
.....

4/ Do you have data for 1000 seed weight of *Cyclamen* sp.? ..... g.

5/ Do you agree with the Pure Seed Definition (N° 10) given by the ISTA Rules 1999 for *Cyclamen* sp? Yes
No, I will suggest.....
.....

6/Can you provide photographs of normal and abnormal seedlings for the handbook?

Yes No

7/Can you provide photographs of growing stages of *Cyclamen* sp seedlings and young plants for the handbook?

Yes No

8/ Do you have any suggestion regarding *Cyclamen* sp lab tests and/or the handbook?

.....
.....
.....
.....
.....

Figure 1. Questionnaire on *Cyclamen* sp.

Report of ISTA FSC *Cyclamen* Working Group
Summary of Questionnaire on *Cyclamen* (October 2001)

By Rita Zecchinelli, ISTA FSC *Cyclamen* Working Group Leader

The questionnaire was sent to 16 members of FSC and reported by 11 of 10 countries. The answers are:

1. Degree of experience on testing *Cyclamen* seeds: 6 laboratories: no experience, 3 laboratories: just a few samples (< 10 samples/year), 2 laboratories: more experience (> 50 samples/year)

2. Frequency of seedlings abnormalities: see the table below

3. Is it necessary to revise the germination methods in ISTA Rules: 1 answer: substrate to be compost, count days 35-49 1 answer: final count 42 days, 2 answers: darkness

4. Do you think that seedling evaluation group A 2.1.4.3 is correct: All answers CORRECT

5.Data for 1000 seed weight of *Cyclamen:* from 4 to 11g, from 5 to 10g, 12 g.

6. Do you think that pure seed definition N° 10 is correct: All answers CORRECT

7. Photographs for abnormal: YES (Italy, France)

8. Photographs for growing stages: YES (Italy, France)

The Report of FSC ISTA Cyclamen Working Group is continued on page 34.

Table 1. The most frequent types of abnormal seedlings:

Type of Abnormality	Frequency in the questionnaire	Frequency in the ring test
I. Primary root		
I.2 Stubby	XXX	XXXX
I.3 Retarded	X	XXX
I.8 Spindly	0	XX
I.12 Decayed	XXX	XX
II. Hypocotyl		
II.2 Not forming a tuber	XX	XX
II.6 Constricted	XX	0
II.10 Spindly	XX	0
II.12 Decayed	XXX	X
III Cotyledons		
III.3 Broken or damaged	XX	0
III.8 Decayed	XXX	X
VII Seedling as a whole		
VII.1 Deformed	XX	XX
VII.4 Two fused together	X	X
VII.7 Spindly	XXX	0
VII.9 Decayed	X	XX

X seldom; XX seldom to regular; XXX regular; XXXX regular to often; XXXXX often

Summary of *Cyclamen* ring test (March 2002)

2 samples of *Cyclamen* seeds - Germination and TZ test

1. Germination ring test

7 participating laboratories in alphabetical order:

- Agri Seed Testing, Oregon - USA (Sharon Davidson)
- GEVES, SNES - F (Sylvie Ducournau)
- ENSE - Italy (Rita Zecchinelli)
- LaRAS - Italy (Enrico Noli)
- Naktuinbow - Netherland (Petra Remeeus)
- National Institute for Agricultural Quality Control - Hungary (Gyöngyi Ivanovics)
- Official Seed Testing for England and Wales - Great Britain (Linda Maile)

Two laboratories carried out the tests both in light and in darkness condition; two laboratories in darkness and three in light only.

Observations

Generally speaking, germination of *Cyclamen* seeds seems to be higher in darkness than in light and the results obtained by the different laboratories are more homogeneous.

The different value of germination between light and dark is due to the number of abnormal seedlings: the light seems to increase the amount of abnormalities. Regarding the kind of abnormal seedlings (shown in Table 1), the ring test confirms the results of the questionnaire and shows that in case of *Cyclamen* the classification of abnormalities regards most of the structures of the seedlings.

The final count for some laboratories was 35 days, but most of them (5) preferred longer duration of the trial, according to ISTA Rules 5.6.4.A Come believed that the duration of 35 days does not fit to the majority of seed lots of *Cyclamen*.

Conclusions

ISTA method for germination is correct. By the results obtained from the referee test the recommended method is TP, 20°C for *Cyclamen*.

ISTA method gives no specification about the light conditions but according our observations germination in dark gives better results and less abnormal seedlings.

It often occurs that normal seedlings can not develop within the 35 days period so final count day can be put 7 days later according to ISTA Rules 5.6.4.A. No statistical evaluation was calculated.

Table 2. Germination test method according to ISTA Rules

Substrate	Temperature (°C)	First count (days)	Final count (days)	Additional directions (breaking dormancy etc.)
TP, BP, S	20, 15	14-21	35	KNO3, soak in water 24 hours

Figure 1 - 3:

Results of germination ring test of *Cyclamen*

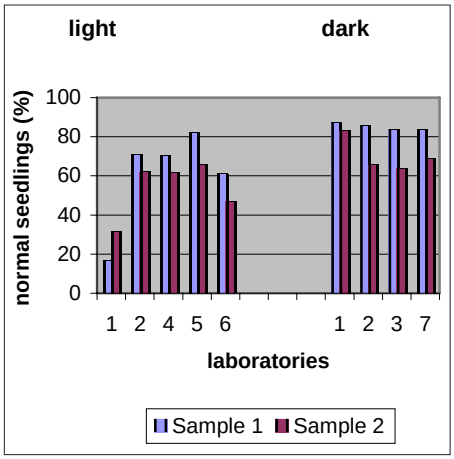


Figure 1. Rate of normal seedlings

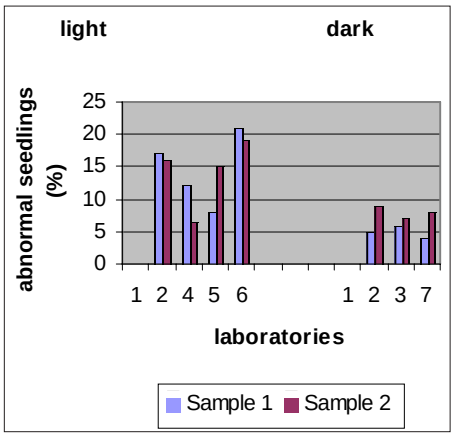


Figure 2. Rate of abnormal seedlings

(Note: laboratory No. 1 reported 0 abnormal seedlings in both tests)

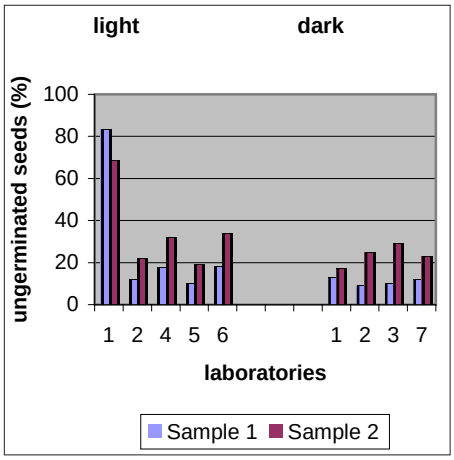


Figure 3. Rate of ungerminated seeds

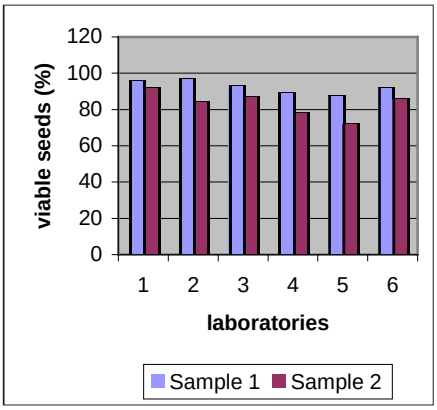
Report of ISTA FSC *Petunia* Working Group

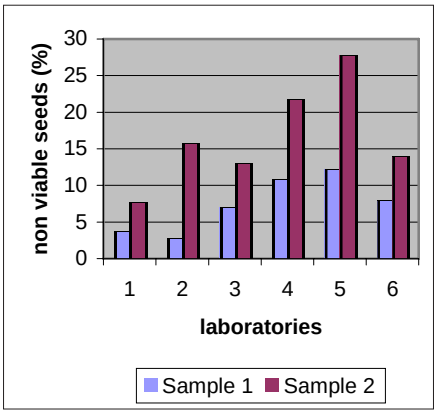
Summary of Questionnaire on *Petunia* (October 2001)

By Sylvie Ducournau, ISTA FSC *Petunia* Working Group Leader

2. Tetrazolium ring test

6 participating laboratories in alphabetical order:
- Agri Seed Testing, Oregon - USA (Sharon Davidson)
- GEVES, SNES - F (Sylvie Ducournau)
- Laboratorio Sementi, MiPAF, ex ASFD-Italy (Fabio Gorian)
- Naktuinbow - Netherland (Petra Remeeus)
- National Institute for Agricultural Quality Control - Hungary (Gyöngyi Ivanovics)
- Official Seed Testing For England and Wales - Great Britain (Linda Maile)





ISTA method:
Pretreatment:
- Soak 24 hours in water at 20°C

Preparation for staining:
- Cut longitudinally through the seed near the hilum

Staining:
- 24 hours, 1% TZ-solution, 30°C

Preparation for evaluation:
- Expose the embryo by slicing the endosperm step by step

Evaluation (maximum area of unstained, flaccid and/or necrotic tissue permitted):
- The position of the embryo inside the endosperm can differ

Conclusions:

The recommended ISTA method is correct. No statistical evaluation was calculated. ■

The questionnaire was sent to 7 persons from 7 countries. The answers are:

1. Frequency of seedling abnormalities. (see table hereafter)
2. Is it necessary to revise the germination methods in ISTA Rules. All the answers are: No
3. Do you think that seedling evaluation group A.2.1.1.1 is correct. All the answers are: Yes
4. Can you provide photographs of normal and abnormal seedlings for the Handbook.
One laboratory can provide photographs, one can try if necessary.
5. Data for 1000 seed weight of *Petunia*. 4 answers quite similar : around 0,1 g (minimum: 0,09 g ; maximum : 0,16 g)

Table 1. The most frequent types of abnormal seedlings

Type of Abnormality	Frequency in the questionnaire	Frequency in the ring test
I. Primary root		
I.1 Stunted	XX	X
I.2 Stubby	0	XX
I.3 Retarded	X	X
I.4 Missing	XXX	XX
I.8 Spindly	0	0
I.11 Glassy	XX	0
II. Hypocotyl		
II.1 Short and thick	XX	X
II.11 Glassy	XX	XXXX
III Cotyledon		
III.2 Deformed	X	0
III.3 Broken or damaged	X	X
III.5 Discoloured	X	0
III.6 Necrotic	X	X
III.8 Decayed (primary infection)	X	X
VII Seedling as a whole		
VII.1 Deformed	XX	X
VII.6 Yellow or white	X	X
VII.7 Spindly	0	0
VII.8 Glassy	XXX	XX
VII.9 Decayed (primary infection)	X	XXX

X seldom ; XX seldom to regular; XXX regular; XXXX regular to often; XXXXX often

Summary of *Petunia* ring test (2002)

3 samples of *Petunia* seeds - Germination test

7 participating laboratories in alphabetical order:

- ENSE- Italy (Rita Zecchinelli)
- GEVES-SNES, France (Sylvie Ducournau)
- Naktuinbouw- Netherlands (Petra Remeeus)
- National Institute for Agricultural Quality Control,- Hungary (Gizi Horvath)
- National Seed Testing Laboratory, - Yugoslavia (Mirjana Milosevic)
- Norvegian Agricultural Inspection Service, Seeds Department, -Norway (Hakon Tangeras)
- Ransom Seed Laboratory- U.S.A. (Aleta Meyr)

(see Table 2. below)

· Substrate: one laboratory uses top of blotter, medium wet

· Temperature: one laboratory uses a non ISTA temperature (25°C)

· Light: one laboratory does not use light for the germination

· Duration: one laboratory has a duration of the germination test of 21 days instead of 14 days

· Pre treatment: 3 laboratories use pre chilling (2 to 5 days) and 2 laboratories use KNO3.

Observations

Pre chilling does not seem to increase the germination rate of samples used in the ring test.

Absence of light during the test does not allow to evaluate defects of cotyledon.

Temperature of 25°C (non ISTA) does not decrease the germination rate of the samples tested.

Deviation of laboratory 06 is difficult to explain. Perhaps it could be due to substrate and/or humidity of substrate.

Conclusions

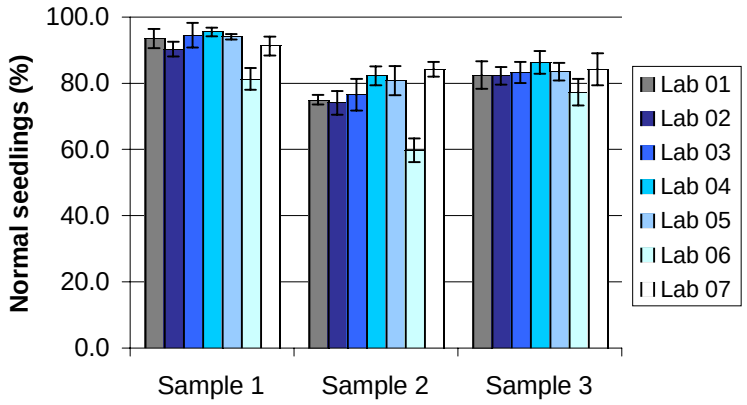
ISTA method for germination is correct. By the results obtained from the referee test the recommended method is TP, 20-30 °C for Petunia.

Statistical evaluation of test results

Germination results have been analysed statistically with a tool developed to assess repeatability and reproducibility in inter-laboratory tests according to ISO 5725-2 (Grégoire S., ISTA Web Site).

· Results are reported sample by sample

· Statistical analysis is reported in the tables below for normal seedlings, abnormal seedlings and ungerminated seeds.



h values superior to h theoric

		H value	Significance level 1%	Significance level 5%
Sample 1	Lab 06	-2.100	1.98	1.71
Sample 2	Lab 06	-2.001		
Sample 3	Lab 06	-1.989		

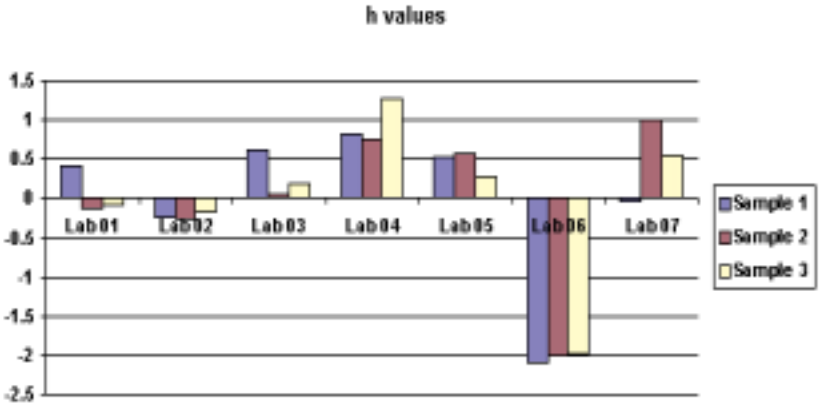


Figure 1. Normal Seedlings

Table 2. Test method according to ISTA Rules

Substrate	Temperature (°C)	First count (days)	Final count (days)	Additional directions (breaking dormancy etc.)
TP	20-30; 20	5-7	14	Prechill, KNO3

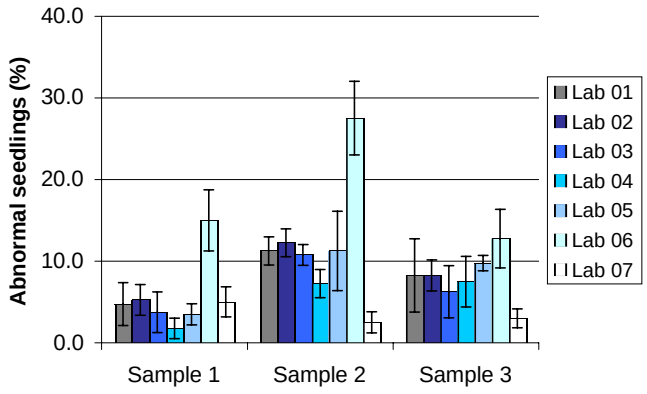
The h values show the tendency for a laboratory to give over estimations or under estimations, compared to the mean of all the results available.The k values show the variability of the repeats.

In general results of normal seedlings are in good accordance between laboratories, except one lab (n°06). If we analyse the results of abnormal seedlings, laboratory 06 reports high quantity of abnormal seedlings with root and hypocotyl defects.

The tendency observed for laboratory 06 to find less normal seedlings and more abnormal seedlings than the overall mean is highly significant.

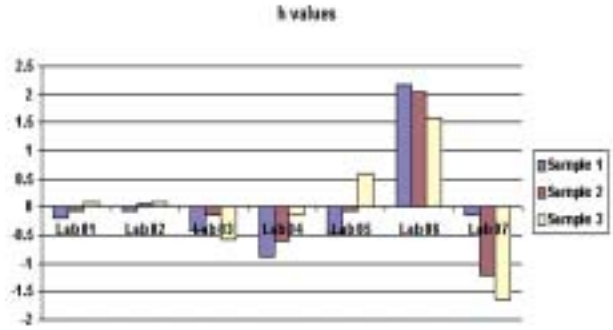
Laboratory 07 has tendency to find less abnormal seedlings than the mean of all labs, but it is not significant.

For the ungerminated seeds, no significant difference has been found between laboratories.



h values superior to h theoric

		H value	Significance level	
			1%	5%
Sample 1	Lab 06	2.180	1.98	1.71
Sample 2	Lab 06	2.036	1.98	1.71



k values superior to k theoric

		K value	Significance level	
			1%	5%
Lab 01	Sample 3	1.551	N.S.	1.55
Lab 05	Sample 2	1.718		
Lab 06	Sample 1	1.620		
Lab 06	Sample 2	1.595		

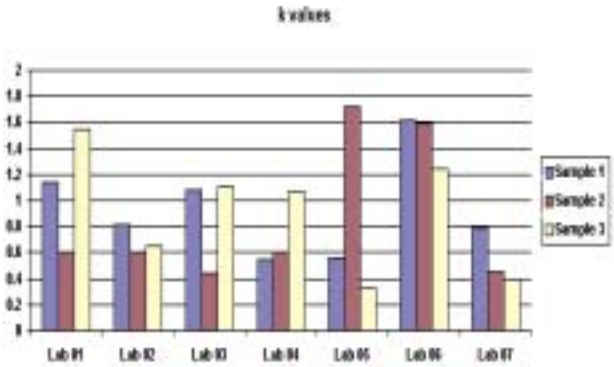
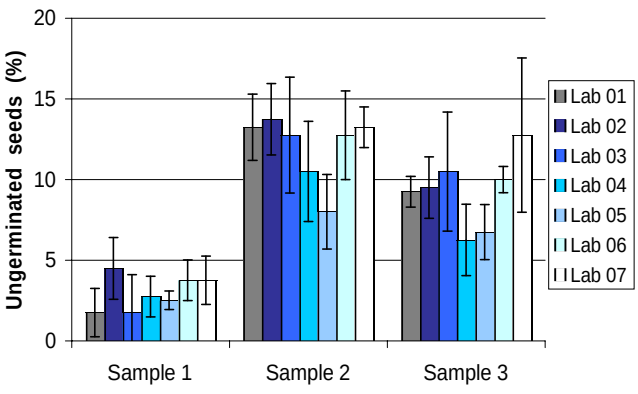
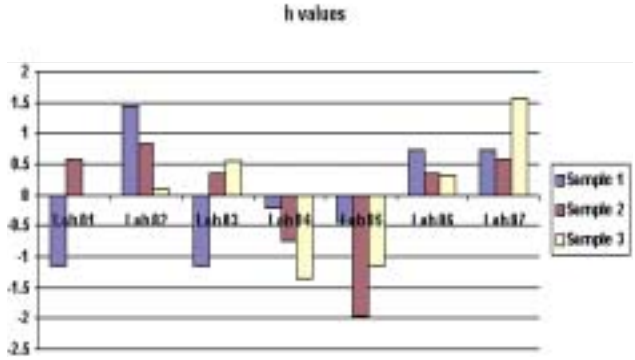


Figure 2. Abnormal seedlings



h values superior to h theoric

		H value	Significance level	
			1%	5%
Sample 2	Lab 05	-1.955		1.55



k values superior to k theoric

		H value	Significance level	
			1%	5%
Lab 07	Sample 3	1.797	1.79	1.55

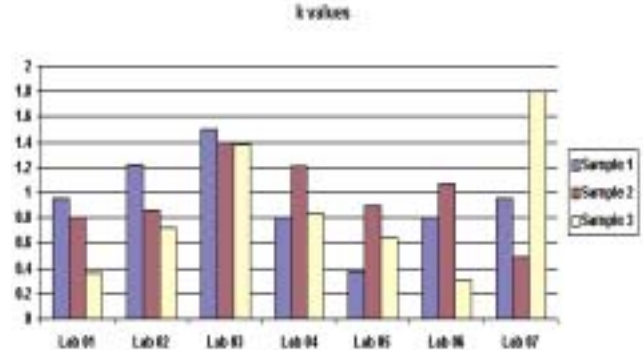


Figure 3. Ungerminated seeds

Qualstat and Seedcalc

Two Programmes available in the ISTA Statistical Toolbox at www.seedtest.org/STA/sta_toolbox.cfm

By Sylvain Grégoire, ISTA Statistics Committee Chair and
Kirk Remund, ISTA Statistics Committee Member

Two computer programs are available in the ISTA statistical toolbox on the web site at http://www.seedtest.org/STA/sta_toolbox.cfm
These programs can help you to:
- check the efficiency of existing control procedures
- select appropriate new control procedures

if your aim is to check if the number or proportion of seeds which are not of the expected common type are within acceptable limits.

If you wish to understand the principles used by Qualstat and Seedcalc, you can read the paper "Statistical considerations in seed purity testing for transgenic traits" in Seed Science Research (2001) 11, 101-119 Remund K., et al.

Both programs work:
if you check each individual seed or plant
or
if you are able to detect unexpected seeds in a group (or pool) of seeds as in ELISA, biochemical or DNA analysis to detect specific traits or bacteria.

In both programs you can see the influence of false positives and false negatives on your decision schemes.
The graphics provide an easy way to see the efficiency of different sampling schemes.
The values of the buyer and seller risk probabilities are provided if you need to put them in the protocols or quality assurance manuals.
Qualstat runs independent of any other software program.
Seedcalc is a spreadsheet that runs in Microsoft Excel®.

Both programs provide designs for standard single and double stage sampling schemes. Qualstat also includes some more complex sequential sampling schemes. Seedcalc allows the user to incorporate some simple calculations to estimate the expected cost of implementing a given sampling scheme.
Qualstat has an accompanying user manual. Seedcalc has an attached sheet with brief user instructions for each calculations sheet. A more detailed users manual is forthcoming for Seedcalc.

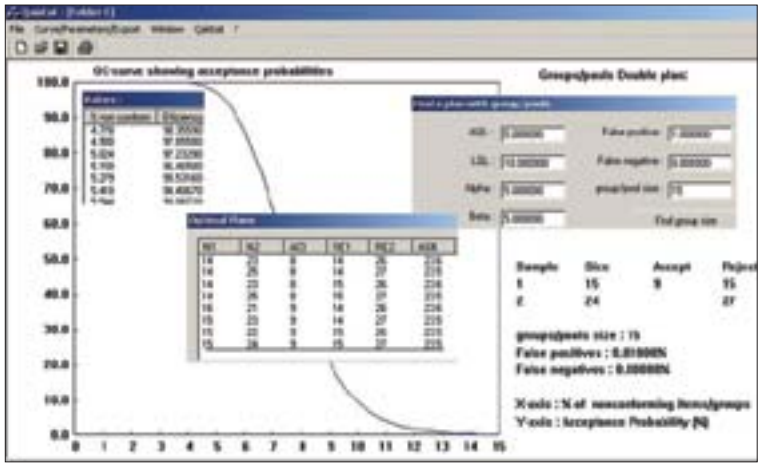


Figure 1. Qualstat

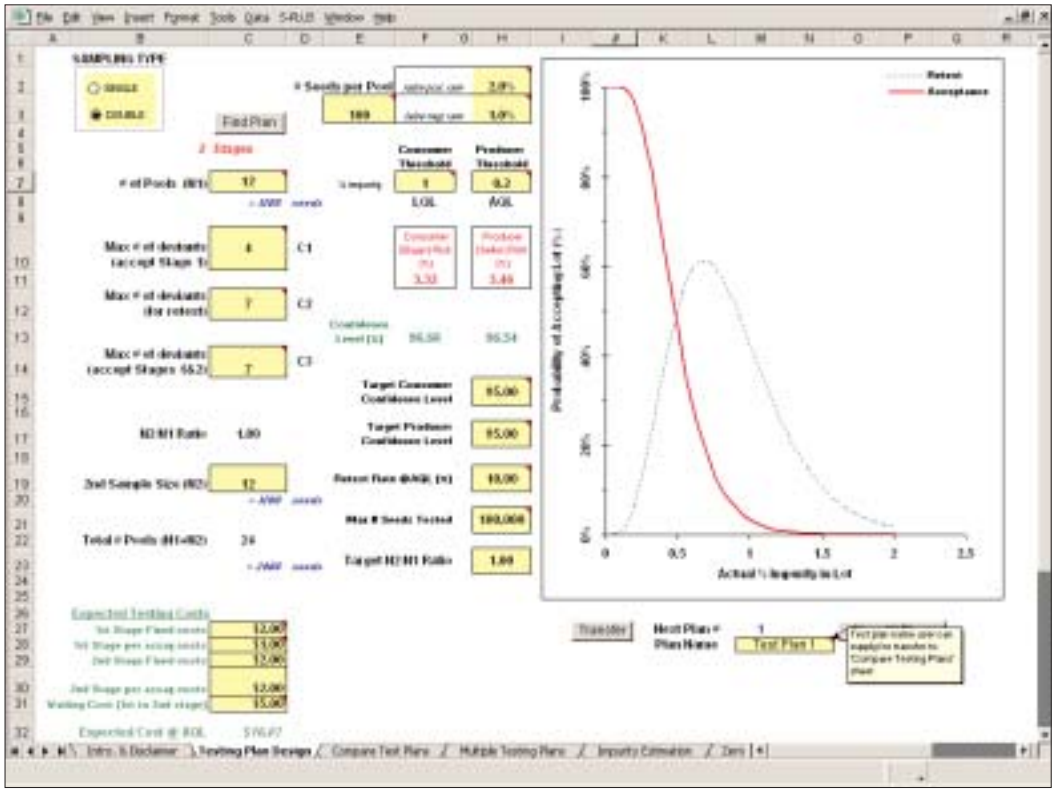


Figure 2. Seedcalc

7th ISTA Tetrazolium Workshop 2002

Karlsruhe-Augustenberg, Germany, July 29 - August 2, 2002

By Adolf Martin Steiner, ISTA Member,
Norbert Leist, ISTA President and
Ronald Don, ISTA Tetrazlioum Committee Vice-chair

Nineteen seed analysts from 17 different countries attended the 7th ISTA Tetrazolium Workshop 2002 hosted by the State Agricultural Extension and Research Station Augustenberg (LUFA), Karlsruhe, Germany. In addition, there was:
- the host Prof. Dr. Norbert Leist, ISTA President, former TEZ Chairman, lecturer and co-organiser;
- the leader of the Workshop Ing. Augusto Martinelli, Chairman of the TEZ;
- co-organisers and lecturers:
Ronald Don, Vice-Chairman of the TEZ and Member of the Executive Committee;
Prof. Dr. Dr. h. c. Adolf Martin Steiner, former Vice-chairman of the TEZ, Honorary Lecturer; and
Dr. Jose Franca Neto, Member of the TEZ.
- Instructors supporting the practical work Mrs. Stephanie Krämer and Helga Werth.

From Augustenberg Mrs. Andrea Jonitz took care of organisational matters together with Mr. Gerhard Lindörfer and Rainer Knoblauch, the latter also being responsible for transportation. There were also numerous other assistants from Augustenberg providing for the needs of the participants and ensuring that the workshop was a success.

The organisers, lecturers and instructors with the experience of the 5th TEZ Workshop in Edinburgh and the 6th TEZ Workshop in Buenos Aires (cf. ISTA News Bulletin 116, 6-9, 1997) had prepared a program designed to achieve the aims of the Workshop:
(i) Adding to uniformity in TEZ testing on an international basis by jointly carrying out tests and discussing their evaluation.
(ii) Deepening the understanding of TEZ testing by increasing participants knowledge of the scientific background to TEZ testing.
(iii) Providing participants the opportunity to comment on the amendments in the ISTA Rules of 1999 and 2002.
(iv) Explaining the working program of the ISTA TEZ Committee and drawing up a list of future activities.

In view of these aims it was a pleasure to welcome colleague Annette Miller the Chair of the TEZ Committee of ISTA's national sister organisation AOSA.

Nineteen seed analysts from 17 different countries attended the 7th ISTA Tetrazolium Workshop 2002 hosted by the State Agricultural Extension and Research Station Augustenberg (LUFA), Karlsruhe, Germany.

As working basis for the participants the Proceedings of the ISTA Tetrazolium Workshop held in 1997 at the OSTS Edinburgh (published by the ISTA) and a folder containing the contents of addresses and instructions for preparation and evaluation of the species being studied in the practical sessions was distributed. In relation to the lectures Prof. Steiner provided handouts that documented the progress since1997, and Vice-Chairman Don provided copies of his excellent photographs with explanations on a CD-ROM. Thanks to the generous support of the Workshop by sponsors, a complete set of tools for TEZ testing contained in a smart leather wallet with the insignia of the Workshop was presented to everyone as a practical and lasting memento.

For practical work, a spacious laboratory was provided together with necessary additional rooms in the same building which was completely reserved for the Workshop. The Zeiss-binoculars and the video demonstration facilities were of the most modern type. The lectures were presented in a hall in the historical Castle Augustenberg. The 200 meters between venues pleasantly allowed participants some time for free discussion whilst on route.

The program of the Workshop as shown in the first insert was compiled as a result of a questionnaire sent to participants. When testing a species, an introduction and final discussion was held by Ing. Martinelli assisted by respective specialists. The lectures, listed in the second insert, were timetabled to fill the gaps between the stages of practical testing. The alternate sessions of practical and intellectual challenge created a stimulating atmosphere.

Special mention has to be made of the unsurpassed hospitality of our host. The participants lodging in two Hotels were transported to and from the workshop and transport was also provided for cultural and social events. This made commuting comfortable and supported discussion and friendship between participants. The Workshop building was situated in an

arboretum neighbouring orchards. The floral displays in the buildings were admired by all and tea/coffee breaks took place in a light filled lobby or under the blue sky in the front of the building. Hot and cold beverages and biscuits were available and each day different tasty local food specialities were presented. Lunch was served in an impressive vault of a historical building 10 minutes walk from the LUFA and this was celebrated every day as another enjoyable get-together. All this contributed to the friendly atmosphere and gave renewed impetus for an afternoon of concentrated and dedicated earnest work.

Several cultural and social highlights enriched the working program. On Sunday, prior to the start of the workshop, a guided walking tour revealed scenic views of the old village of Durlach which was founded in 1161. At the subsequent "Meeting in the Biergarten Traube" those having arrived too late for the walking tour joined the others for a typical meal accompanied by some traditional refreshments. On Monday there was an evening tour to Karlsruhe, which was the residence and capital of the former Great Dukedom Baden. There we saw the Botanical Garden, the Castle, the Supreme Court Buildings, the shopping mall "Kaiserstraße" and the Market Place. The day ended with a meal at one large table in an old hall of a brewery. An invitation to Rainer's cottage at Grötzingen followed on Tuesday. After a humorous address by Prof. Leist and a kind welcome by Mr. Rainer Knoblauch, in his back garden, Mrs. Alice Leist and Mrs. Birgit Klauke were introduced as those being responsible for this event. The yard and an old barn had been set out with tables and chairs. Tasty salads, well-seasoned baked or grilled sausages, fresh vegetables, local breads and sweet desserts delightfully combined with soft drinks, beers and local wines provided everyone with a meal and a night to remember. Some ate in the garden under starlight and some in the barn with candle light and when the meal was over the ISTA family came together for an unforgettable sing song. Our most cordial warm thanks must go to the generous hosts. Wednesday evening or what was left of it after a marathon practical session was at own disposal.

Participants were then treated by Prof. Steiner to a guided tour of the 35 hectare Hohenheim Gardens. Part of these form the famous State Arboretum and contains about 2500 different tree species and varieties. About two dozens of the trees are more than 230 years old having been planted by Duke Carl Eugen of Württemberg, the founder of the Gardens in 1776.

On Thursday morning we set out for an excursion to the University of Hohenheim. On arrival in the Institute of Plant Breeding, Seed Science and Population Genetics, after a refreshment and a snack, Prof. Steiner presented a lecture on "The History of Tetrazolium Testing". Thereafter participants were treated to an exhibition of historical tools, equipment, drawings and photographs. These were from the times of Prof. Friedrich Nobbe, who founded seed testing in 1869, and of early TEZ testing founded by Prof. Georg Lakon in 1942. Following a tour through the Hohenheim German Agricultural Museum lunch was taken at the students refectory. Participants were then treated by Prof. Steiner to a guided tour of the 35 hectare Hohenheim Gardens. Part of these form the famous State Arboretum and contains about 2500 different tree species and varieties. About two dozens of the trees are more than 230 years old having been planted by Duke Carl Eugen of Württemberg, the founder of the Gardens in 1776. There was the unique possibility to compare an established 230 years old garden with a recently planted enlargement. Finally, in one of the conserved historical buildings, contained in the gardens, the Small Museum of the History of Hohenheim was visited. Hohenheim was first mentioned in 1100.

On the route back to Karlsruhe, the roman style Cistercian Monastery of Maulbronn, founded in 1147, was visited. This is the best preserved German Monastery and is registered as an UNESCO World Cultural Heritage Monument. An excellent guide demonstrated why this monastery receives more than 200.000 visitors a year. The tour ended with a Festive Workshop Dinner in the "Monastery tap-room" housed in one of the huge historic buildings.

At this dinner Prof. Steiner and Mrs. Werth, who were both going to retire this year, were honoured and said farewell. With it 60 years of TEZ research at Hohenheim will end. It had started with Prof. Georg Lakon who invented TEZ testing in 1942. He was followed by Prof. Werner Lindenbein and Dr. Helene Bulat who established Chapter 6 in the ISTA Rules in 1965, and they were followed by Prof. Steiner who revised Chapter 6 in 1999. Now, in Germany Augustenberg takes over the lead in TEZ testing and research and, hence, organised this 7th Tetrazolium Workshop to honour Hohenheim's achievements.

After five days of:
- earnest work and enthusiastic discussion on tetrazolium testing, of
- delightful cultural and social events and
- renewed and new friendships,

every participant received an ISTA Certificate of Participation.

Special thanks were expressed to the organisers and lecturers by presenting each one a unique small hand-painted porcelain plate. This showed a painting of the old Augustenberg Castle, and was inscribed with the recipients name, Seed Testing Station Augustenberg and ISTA-Tetrazolium-Workshop. The gifted artist was Mrs. Gerda Jonitz, sen.

For sure, nobody will ever forget the experience gained by the daily work striving for uniformity in TEZ testing and nobody will forget the friendly hours spent with the colleagues. Hopefully, many more of such sci-

entifically and personally rewarding Workshops are to come. Wholeheartedly, warm thanks again to the hosts, the organisers and lecturers, thanks also to the participants.

Finally, many thanks to Prof. Dr. Friedel Timmermann, Director of the LUFA Augustenberg, for his continuous support of the activities of the Section Seed Testing and Applied Botany. Without such support the Workshop would not have been possible. ■

Program of the 7th ISTA Tetrazolium Workshop 2002

1st day	Welcome and introduction *Lolium* sp., *Hordeum vulgare*; preparation, staining and evaluation; lectures
2nd day	*Tagetes* sp., *Zea mays*, *Lotus* sp., *Lycopersicon esculentum*; preparation and staining; lectures; evaluation of parallel germination test *Zea mays*; germination test versus viability test in *Zea mays*
3rd day	*Tagetes* sp., *Zea mays*, *Lotus* sp., *Lycopersicon esculentum*, *Oryza sativa*; evaluation; lectures; guided tour LUFA Augustenberg Seed Testing Station; *Glycine max* evaluation; lecture
4th day	Excursion to the University of Hohenheim
5th day	Finishing practical work, lectures, discussion forum; Farewell and workshop closure

Lectures at the 7th ISTA Tetrazolium Workshop 2002

Presentation of the International Seed Testing Association ISTA.
N. Leist
Presentation of the ISTA Tetrazolium Committee 2001 - 2004.
A. Martinelli
Tetrazolium salts and biochemistry of tetrazolium reduction.
A. M. Steiner
Presentation of the 2001 and 2002 amendments to the 1999 edition of the ISTA Rules in the Annexe to Chapter 6.
A. Martinelli
Relationship between germination testing and viability testing.
A. M. Steiner
Explanations of staining patterns caused by heat damage, water damage, mechanical damage, freeze injury, micro organisms etc.
R. Don
Presentation of the new ISTA Tetrazolium Handbook.
N. Leist
Quality assurance and health and safety in tetrazolium testing.
R. Don
Tetrazolium testing in Glycine max.
J. Franca Neto
History of topographical tetrazolium testing.
A. M. Steiner
Tetrazolium testing: sample size, statistical basis for assessing tolerances and use of sequential testing procedures.
A. M. Steiner and R. Don
Tetrazolium staining as an indicator of vigour.
A. M. Steiner and R. Don

ISTA/ICPP Seed Pathology Workshop 2003

Lincoln University, Canterbury, New Zealand Feb. 10-13, 2003

By Jim Sheppard, ISTA Plant Disease Committee Chair

The 8th International Congress of Plant pathology held in Christchurch, New Zealand provided an opportunity for the Plant Disease Committee of ISTA to conduct a seed pathology workshop following the Congress at Lincoln University. This was the first ever ISTA workshop in New Zealand and the first ISTA gathering in New Zealand since the Fifteenth ISTA Congress in 1968.

Twenty-five people registered for the workshop. Five were from Australia, 4 from UK, 3 from USA, 3 from New Zealand, 2 from Denmark, 2 from India, and one each from Norway, Israel, The Netherlands, Spain, Canada and Switzerland. Eight current members of the PDC (Jim Sheppard, Valerie Cockerell, Guro Brodal, Steve Roberts, Denis McGee, Kees Langerak, Rivka Hadas, and Rouke Bakker) were at the workshop. Presenters were assisted by two postgraduate students (from Brazil and Uruguay), Dr. Wadia Kandula and Mr Rouke Bakker who also participated. The workshop concentrated on detection of seed-borne diseases of vegetables and other crops of interest to the Asia/Pacific region. Participants had the opportunity for practical "hands-on" training, as well as information transfer provided by ISTA PDC members and other seed health specialists.

Daily sessions were composed of laboratory exercises interspersed with oral presentations on various topics pertaining to seed health testing. Methods for detection of several host/pathogen combinations were demonstrated. *Xanthomonas campestris* pv *campestris* (blackrot) of Crucifers (Steve Roberts). This method has been the subject of extensive development and review is proposed for inclusion into the International Rules for Seed Testing this year. Jim Sheppard demonstrated a recent comparative test for detection of *Phoma lingam* (blackleg) of Crucifers using the current ISTA method, 7-004, and proposed modifications using freezing blotters or absic acid. The results from the various treatments were compared. Guro Brodal (Norway) presented the Osmotic blotter test for detection of *Drechslera* spp. in barley and oats used in the Nordic countries for many years. Rouke Bakker demonstrated a method routinely used by Biolinc for detection of *Pseduomonas syringae* pv.pisi. The advantages and disadvanges of three agar methods for detection of *Michrodochium nivale* and various *Fusarium* species was examined in a session led by Valerie Cockerell who organized a recent comparative test on these pathogens. Pea seed borne mosaic virus (PSbMV) and lettuce mosaic virus (LMV) are important seed-borne diseases affecting seed production. There is no standard testing method currently available for PSMV but an ELISA method has been adopted by Biolinc. An ELISA method has also been the subject of recent comparative testing. Demonstration of both methods in the laboratory and a discussion of issues related to the suitability of the two tests for routine testing was led by Rouke Bakker. Wadia Kandula present methods described for detection of *Ascochyta* in chickpea and the *Ascochyta* complex in pea and discussed recent advances in molecular methods for detection of these pathogens.

Other topics discussed during the workshop included the role of ISTA in establishing uniformity in seed health testing, molecular techniques in seed health testing, seed health issues for the Asia/Pacific region, and the ISTA quality assurance, accreditation, proficiency test and method validation programmes as they relate to seed health testing.

The learning experiences and the social interaction that occurred made this a highly successful workshop. It gave organisers and participants the opportunity to put faces to names, and to reassure themselves that their work was up to international standards. The opportunities taken for networking will be invaluable.

On the behalf of the PDC I would like to thank the NZSTI, and particularly Director Professor Murray Hill for supporting the workshop in so many ways. John Hampton for his courage and perseverance in hosting this workshop following a major event such as the ICPP; the dedication and hard Mr Rouke Bakker (PDC member) and Dr Wadia Kandula and the post graduate students. Without their efforts (and long hours) we would not have had such a successful workshop. I would like also to thank my fellow PDC members for assisting with the programme development, oral presentations and laboratory demonstrations.

A proceedings of the workshop will be published by ISTA and many of the presentations can be found on the ISTA online Website at http://www.seedtest.org/pdc/works_p.cfm. ■

Comments from the participants indicate that they found this workshop informative, interesting and interactive.

"The presentations made by the different speakers were excellent".

"A great opportunity for me and I am glad I came".

"Good balance between theoretical information and practical work".

"It was a good idea to include fungi, bacteria and viruses in the same workshop".

"BBQ and dinner were superb. No room for improvement in your kind hospitality".

"The workshop was interesting, well organised, and speakers were well chosen".

"Excellent chance to interact; overall an extremely useful workshop".

"A very productive workshop".

"An entirely enjoyable experience".

1st Announcement - ISTA Forest Tree and Shrub Seed Workshop

October 20 - 25, 2003
Prague, Czech Republic

Zdenka Procházková, ISTA Forest Tree & Shrub Seed Committee Chair

Objectives
The workshop will deal with practical problems related to tree seed testing of both conifer and broadleaf species. The general aim of the workshop is to create discussion and exchange of information in this area. Based on input from the preliminary registration, the workshop will cover all fields of seed testing such as Purity, Germination, Tetrazolium, Health, Excised embryo, Moisture content and X-ray tests; also, other fields of interest are Referee tests, Quality Management System, Precision of test results, Retesting frequencies of stored seeds and Practical work in the laboratory.

Tentative Programme
From Monday, October 20, to Wednesday, October 22nd - presentations, lectures, practical "training"; and a visit to the Central Institute for Supervising and Testing in Agriculture in Prague (CZDL03). Registration for the workshop is scheduled for Sunday evening, October 19, and Monday morning, October 20.

There will be two alternative post-meeting trips (from Thursday, October 23, to Saturday, October 25):
1. Visit to the State Tree Seed Centre in Tyniste nad Orlici (1day bus trip of about 300 km).
2. Visit to the Seed Testing Laboratory for Forest Tree Seeds in Uherske Hradiste (CZDL02), southeastern Czech Republic, and then continue on to visit the Forest Seed Testing Laboratory (SKDL02) in Liptovsky Hradok, Republic of Slovakia (3 day bus trip of about 1 000 km).

Venue
The workshop will take place at the Congress Conference Centre Floret located in the peaceful village Pruhonice, a suburb of Prague, Czech. Pruhonice is situated in a quiet area on the main eastern highway leading to Brno and Vienna, only a few minutes by car from the Prague city centre and within the Prague municipal bus and metro (subway) systems. In Pruhonice there is a beautiful castle "Pruhonice Chateau" which presides over the Botanical Park, now protected by UNESCO.

Instructions for the paper presentations
Participants wishing to make a presentation are asked to submit an abstract of their paper. The papers have to be relevant to the Workshop objectives.
The Abstract includes: Title, author(s), address and text. Title should be CAPITALISED and bold. Authors should be listed in upper and lower case, with initials before the surname. The address should start in a separate line; include E-mail address of the presenting author. The text should not exceed 250 words. Use MS Word 95 or later edition; Paper size A4; Margins 3.0 cm (top and bottom) and 2.5 cm (left and right); Font: Times New Roman, Font size: 9pt. for the entire Abstract.

Please submit an Abstract for oral presentations (not more than 250 words) before 30 June 2003 via e-mail to Zdenka Procházková (Prochazkova@vulhmuh.cz).

For your registration, travel information and hotel reservation please visit the ISTA website or contact:
Zdenka PROCHÁZKOVÁ
FGMRI RS Uherske Hradiste
686 04 Kunovice
Czech Republic
e-mail: Prochazkova@vulhmuh.cz

ISTA Purity Workshop

June 11 - 13, 2003
Seattle, USA

Ken Allison,
ISTA Purity Committee Chair

Workshop content

Identification Sessions:
* *Poa, Bromus, Centaurea, Lolium* and *Festuca*

Quality Assurance Topics:
* Blind testing of analysts
* Training personnel
* Monitoring of purity equipment

Discussion Topics:
* Purity concerns for harmonization

ISTA/AOSA Rules
* New rules and proposals for the future

Registration

The workshop will begin at 1.00 pm on Wednesday 11th and finish at 12.00 pm on Friday 13th June. The cost is US$150 per person. Registration deadline is May 10th, 2003!

Hotel reservations

Please make reservations directly at Double Tree Guest Suites in Seattle, WA. Phone +1-206-575-8220, ask for in-house reservation. Be sure to tell them you are with the AOSA/SCST Annual Meeting to take advantage of the group discount rate of 119 US$ + Tax per night.

To register online please go to
www.seedtest.org

1st ISTA Moisture Workshop

Lyngby, Denmark
November 3 - 7, 2003

Harry Nijënstein, ISTA Moisture Committee Chair, and
Jette Nydam, ISTA Moisture Committe Member

General

The ISTA Moisture Committee and the Danish Plant Directorate have the pleasure to invite you to their Workshop on Moisture Testing.

Scope

The aim of the workshop is to create discussion and exchange of information in the field of moisture testing.

Programme

Lectures and discussions
- Background to seed moisture
- Quality Assurance with reference to moisture testing
- Future of moisture testing
- New species and methods (method validation)
- Development of handbook for moisture testing

Demonstrations and excursions
- Moisture meters, Danish Grain Network
- Excursion to seed company and to manufacturer of moisture meters

Practical work
- Oven test (soft wheat test)
- Calibration of moisture meters

Registration

The number of participants is limited to 20. The provisional registration costs are EUR 250, which includes participation, handouts, refreshments during breaks, lunches, daily travel from hotel to laboratory. The costs for a single hotel room including breakfast will be around EUR 100 per night. The deadline for registration is August 1, 2003.

Registration Form

Title ……	Position ……
Name ……..	First name …….....
Company / Institute…….....	Address ……..……
Postal Code …….....	City ……
Phone number …….....	Country ……..…....
Email ……..	Fax Number ……..

Return this form to

ISTA Secretariat
Zürichstrasse 50
P.O. Box 308
8303 Bassersdorf, Switzerland
Email: ista.office@ista.ch
Fax: +41 1 838 6001

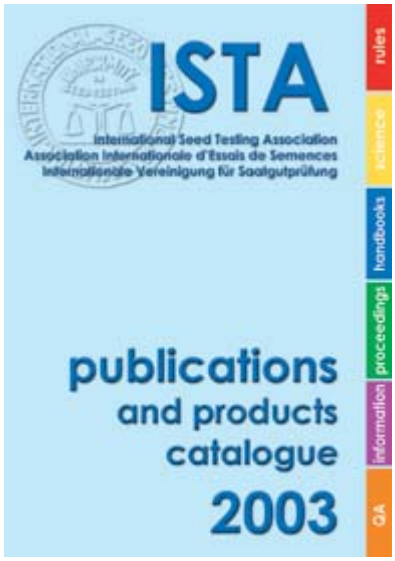
stay up to date...

The New ISTA Publications Catalogue 2003 and the ISTA Workshops Programme 2003 are both available from the ISTA Secretariat, free of charge.

If you have not yet received these, please contact the Secretariat, and have them forwarded to you.

Or visit ISTA OnLine at

www.seedtest.org



Proficiency Test Committee

Call for Test Leaders

By Doug Ashton, ISTA Proficiency Test Committee Chair

The Proficiency Test Committee invites participation by anyone interested in becoming a proficiency test leader. This includes any who have previously been leaders as well as those with no experience in preparation of proficiency test samples.

The schedule for the proficiency testing programme is finalised during each triennial Congress. The three year plan includes the list of species for nine rounds, the tests which will be conducted (purity, other seed determination, germination), and the names of the test leaders who have volunteered for the rounds. Each round leader is responsible for finding three seed lots having a range of quality for germination and which are reasonably free from impurities. If the tests include other seed determination, the leader develops a list of impurities to be added, which is approved by the Committee Chair. The test leader prepares approximately 150 sub-samples from each seed lot and conducts homogeneity tests before distribution. The ISTA Secretariat carries much of the burden of preparing the written communications, distributing the samples, entering data into the database and preparing the reports. The test leader reviews data summaries prepared by the Secretariat and writes recommendations for those laboratories which have demonstrated difficulties with the proficiency test. The leader advises the Secretariat on any questions concerning the round and may receive requests for assistance from participating laboratories which experienced specific problems. Test leaders are provided with a detailed protocol for sample preparation and are assured advice and support from the Secretariat and Committee. Some of the costs associated with sample preparation, (e.g. purchase of seed lots, containers, shipping, etc.), are reimbursed by the Secretariat, but the cost of labour cannot be reimbursed. At this time, the proficiency testing programme is focused on purity and germination testing; however, pilot studies are underway or being considered for vigour, moisture, seed health, tetrazolium and tree seeds, so it is likely that in the near future leaders will also be needed for these fields of testing.

Although there is a significant time commitment, previous test leaders will confirm that this is a very rewarding experience because it provides an insight into the complexities of proficiency testing and gives a direct behind-the-scenes view of a very important ISTA activity. If you are interested in becoming a proficiency test leader, or would like to receive more information, please contact Martina Rösch at the Secretariat. The new Committee will be formed prior to the next Congress in Budapest in May 2004. ■

ISTA International Rules for Seed Testing

Announcement of the new Edition 2003

ISTA's primary instrument in promoting uniformity in seed testing procedures is the 'International Rules for Seed Testing', which lays down detailed standard techniques and procedures. The publication includes 17 Chapters and Appendices describing principles and definitions in detail, assisted by many tables and the methods to be used. The 'International Rules for Seed Testing' is designed for the principal crop species of the world, but apply in general, if not in detail, to any species of crop plant, even including those not mentioned in the text.

The 'International Rules for Seed Testing' is approved and amended at ISTA Ordinary and Extraordinary Meetings on the basis of advice tendered by the ISTA Technical Committees. The Edition 2003 includes the latest changes, which were voted on at the ISTA Extraordinary Meeting 2002, held in Santa Cruz (Bolivia) from July 3 - 6.

Explanatory note: The complete set of the 'International Rules for Seed Testing' as per January 1, 2003, will include the following two separate publications: International Rules for Seed Testing, Edition 2003 and Annexe to Chapter 7, Seed Health Testing Methods.

What is new about the 'International Rules for Seed Testing'?

The Edition 2003 will cometo you in a binder. Each year, updates, in the form of a 'single paper collection' including additions or replacements of existing pages will be published, and could then be separately inserted into the binder. With this system ISTA will be able to provide more flexibility, enabling faster updates and improvements of the International Rules for Seed Testing. ■

Valid from
January 1, 2003
ISBN:3-906549-38-0
(Available in English)

The price, including the
Annexe to Chapter 7:
CHF 350.-
(Approx. US$ 215.-)

**As part of the ISTA membership services, ISTA Members will receive one free copy of the Rules 2003.*

Advanced Quality Management

Report on the Workshop in Ljubljana, Slovenia, November 11 - 15, 2002

By Martina Rösch, ISTA Accreditation Department

ISTA held its workshop on Advanced Quality Management (QM) in November 2002. This workshop primarily addressed experienced quality system managers who have already participated in one of the previous workshops and/or have set up a quality management system in their organisation. The purpose of this workshop was to deepen knowledge about QM and initiate lively discussions among the participants and the exchange of ideas.

The workshop was being held at the Agricultural Institute of Slovenia (AIS) in Ljubljana, November 11-15, 2002. It was presented by Prof. Dr. Norbert Leist, ISTA President and Heinz Schmid, The Essential Quality Management.

27 participants attended the workshop from 16 different countries. The workshop programme was made up of the preliminary programme and suggestions brought in by the participants prior to the workshop. At the very beginning the participants were requested to formulate their aims and questions so that their needs could be adequately met.

Through the lectures the participants learnt more about theoretical aspects of quality management like internal auditing, QM tools and their application, basic principles of communication and staff motivation. For the technical part many practical examples were presented concerning e.g. calibration of laboratory equipment and sampling of seed lots. The workshop did not only focus on theory but the lectures were supplemented by practicing the newly acquired knowledge in group work. These group work sessions were also a good opportunity to exchange individual experiences made with quality management in small groups.

Also due to the efforts put into the organisation of this workshop by Romana Rutar, head of the seed testing laboratory of the AIS and her team, the workshop was a great success and the participants went home with a suitcase full of new ideas.

Re-accredited ISTA Member Laboratories

Up until February 15, 2003

CH - Switzerland

CHDL01

Eidgenössische Forschungsanstalt für Agrarökologie und Landbau FAL
Gruppe Saatgutqualität

Reckenholzstrasse 191/211
Postfach 412
8046 Zürich
CH-Switzerland

Phone: +41 1 3777111
Fax: +41 1 3777201
E-mail: silvia.zanetti@fal.admin.ch

GR - Greece

GRDL01

Seed Testing Station of Athens
Ministry of Agriculture

Antheon 2, Marousi
151 23 Athens
GR-Greece

Phone: +30 10 6830471
Fax: +30 10 6830917
E-mail: an2u001@minagric.gr

Quality Assurance

The following Quality Assurance Documents are available directly from the ISTA Secretariat.

1. **ISTA Seed Testing Laboratory Accreditation Standard**, Version 3.1, 2002
2. **Guidelines for becoming an ISTA Accredited Member laboratory**, Version 1.1, 2002
3. **Guidelines for Monitoring ISTA Accredited Company Seed Samplers and Company Laboratories**, Version 1.0, 2002
4. **Procedures for Termination, Suspension and Withdrawl of ISTA Accreditation**, Version 1.0, 2002
5. **Guidelines for developing Quality Documention**, Version 1.0, 2002

Seed Trade Association of Kenya

Report on the Accreditation Workshop in Nairobi, Kenya, December 9 - 10, 2002

By Gerhard Schuon, ISTA Accreditation Department

KEPHIS, which is also the Designated Authority in Kenya, supports and recognizes ISTA Accreditation of private laboratories in Kenya.

Following an invitation from the Seed Trade Association of Kenya (STAK) I participated as second resource person, together with Mr Dagallier from OECD in the accreditation workshop held at the Nairobi Hilton in Kenya. Participants included representatives from Ministries of Agriculture, Universities and Seed Trade from Kenya, Tanzania and Uganda. The purpose of the workshop was twofold:

-To explore the possibilities of the Designated Authority of Kenya, delegating some elements of its supervisory activities to the private sector, and
-To look into requirements of quality management systems as far as documentation and quality manuals are concerned.
This second purpose of the workshop was merely touched upon, as the printout from the two previous sessions was distributed and it was felt that this issue had already been covered in depth.

In a number of presentations various aspects of accreditation and certification were looked at. It soon became apparent, that there was some confusion concerning the different concepts applied in conformity assessments. During the workshop these issues could be clarified and all participants reached a common understanding.

Looking at the situation in Kenya, it appeared that there was some potential for partly delegating specific tasks to the private sector. Field inspection and seed testing were identified as two major components that would qualify for being carried out by the private sector under close supervision by government authorities.

ISTA Accreditation pertains to the technical and managerial competence in a seed testing laboratory. As a consequence, accreditation is granted to the laboratory, not to individuals and, following changes to the ISTA Constitution in 1995, this applies to laboratories from both public and private sector. Initially, only private independent laboratories were taken into consideration, but now company laboratories, too, can be accredited. One of the two ISTA member laboratories in Kenya is currently in the process of being re-accredited. It operates under the Ministry of Agriculture and is part of Kenya Plant Health Inspectorate Service (KEPHIS). KEPHIS, which is also the Designated Authority according to national legislation, is willing to support and recognize ISTA Accreditation of private laboratories in Kenya.

Similarly, field inspection along the lines of the OECD Seed Schemes could be carried out by recognized individuals under contract of or employed by members of the private sector. The recognition would be to be governed by certain educational, training and expertise requirements set up by KEPHIS in consultation with other interested parties. A presentation from Dr Muasya from Moi University Nairobi outlined a prospective training programme for candidates. It had been developed together with government representatives to be a starting point in further building up a comprehensive system to meet the requirements of farmers, industry and authorities. Details still have to be worked out; especially who would qualify for official recognition, how and to what extent monitoring will be exerted.

The workshop was a considerable success, bringing most players in the region together and indicating the potential for future development. It shed light on both limitations and possibilities of forthcoming cooperation. Still, a lot of work lies ahead and only future will show whether the private sector will take up some of the activities under governmental supervision and which pitfalls the process will reveal. ■

Seed Testing in Hungary

By Katalin Ertsey, ISTA 2nd Vice President

The next (27th) ISTA Congress will be held in Budapest in the Capital and the economic, financial and cultural centre of Hungary.

Hungary is situated in Central-Eastern Europe, surrounded by the Carpathians and the Alps. The neighbouring countries are Austria, Slovakia, Ukraine, Romania, former Yugoslavia, Croatia and Slovenia.

67 % of Hungary's territory, about 4,7-4,9 million ha, is suitable for agricultural production. The main crops are maize, winter wheat, winter and spring barley, sunflower, potato and other crops as large and small seed of legumes, grasses, oilseed rape, vegetables and so on. The seed sector has an important and valuable role in the Hungarian plant production.

In the context of this activity the inspected seed multiplication acreage and the quantity of certified seed, Hungary has become stable in the last 10 years.

The seed lots moving in the international trade are, according to the last official OECD report:

USA	29 %
Hungary	15 %
France	8 %
Chile	8 %
Italy	6 %
New Zealand	5 %
Australia	4 %
Czech Republic	4 %
Argentina	3 %
Others	18 %

The value of the Hungarian seed export is between 58 - 65 million USD on average per year.

Of course such a strong seed industry requires excellent seed testing with highly educated experts and seed analysts.

Year	Inspected Area	
	ha	%
1992	169.993	100,0
1993	158.944	93,5
1994	180,287	106,1
1995	181.975	107,1
1996	160.457	94,4
1997	155.775	91,6
1998	180.364	106,1
1999	169.142	99,5
2000	156.234	91,9
2001	173.405	102,0
2002	161.018	94,7

Figure 1. Inspected Area

Year	Seed	Potato	Total	%
	certified quantities			
	tons	tons	tons	
1992	306.208	32.533	338.741	100,0
1993	300.535	28.670	329.205	97,2
1994	344.464	34.502	378.964	118,7
1995	323.273	35.348	358.621	105,9
1996	316.301	29.443	345.744	102,1
1997	354.009	19.986	373.995	110,4
1998	335.007	24.314	359.321	106,1
1999	314.512	25.158	339.670	100,3
2000	372.020	26.176	398.196	117,6
2001	340.253	22.383	362.636	107,1
2002	348.383	22.900	371.283	109,6

Figure 2. Certified Seed

Hungarian seed testing has a long history to it. Hungary has been a member of European and international organisations either since their foundation or since the demand for it increased.

1921 - European Seed Testing Association
1924 - International Seed Testing Association

The institutional history of the Hungarian seed testing laboratory:

1895	Royal Seed Testing Station, Budapest
1949	National Institute for Agricultural Quality Control
1951	National Institute for Seed Testing
1952	National Inspectorate for Seeds
1976	National Inspectorate for Seeds and Propagating Materials
1983	Institute for Plant Production Quality Control
1988	Institute for Agricultural Quality Control
1994	National Institute for Agricultural Quality Control

Now the Seed Certifying Agency is a part of the National Institute for Agricultural Quality Control and its task, among others, the official seed testing in Hungary.

The only ISTA Accredited Seed Testing Laboratory is located in Budapest.

The Seed Testing Laboratory works according to a Quality Assurance System, and was one of the first accredited labs under the new ISTA system.

The times of ISTA accreditation were: 30th October 1998 and 20th November 2001.

The central ISTA Laboratory and the other 6 local labs fulfil the "General requirements for the competence of testing and calibra-

tions laboratories ISO/IEC 17025:1999" as well. The tested samples included more than 200 varieties from cereals to flower and medical seeds.

The ISTA laboratory of Budapest handles the ISTA samples and most of vegetable, flowe and medicine seed tests, which demand a well developed technical environment.

Number of tested samples in year 2002:

Analytical purity	25 748 samples
Genetic purity	1 377 samples
Germination	14 701 samples
Seed health	9 997 samples
Moisture content	270 samples

(germination includes TTC and vigour tests)

The staff is highly educated with several experts holding master degrees or more. 22 seed analysts work in the ISTA lab. The lab has different training programs for the company specialists, and an organiser in practical education for the university of agriculture.

Our Hungarian predecessor colleagues worked earlier in different ISTA Technical Committees, obtaining good professional appreciation, and this trend continues now as we have members in the Flower Seed and Tetrazolium Committees, as well as in the Executive Committee.

The ISTA Laboratory in Budapest issues more than 7000 ISTA Certificates yearly. The regional labs work for the Hungarian market, which are accredited by the Hungarian National Accreditation Body. The number of ISTA samplers is 56.

We are looking forward to host a large number of seed experts in May 2004 on the occasion of the 27th ISTA Congress. The scientific program for the Seed Symposium, challenges of the new tendencies, cultural programs and the excursions promise a nice time for all participants. ■

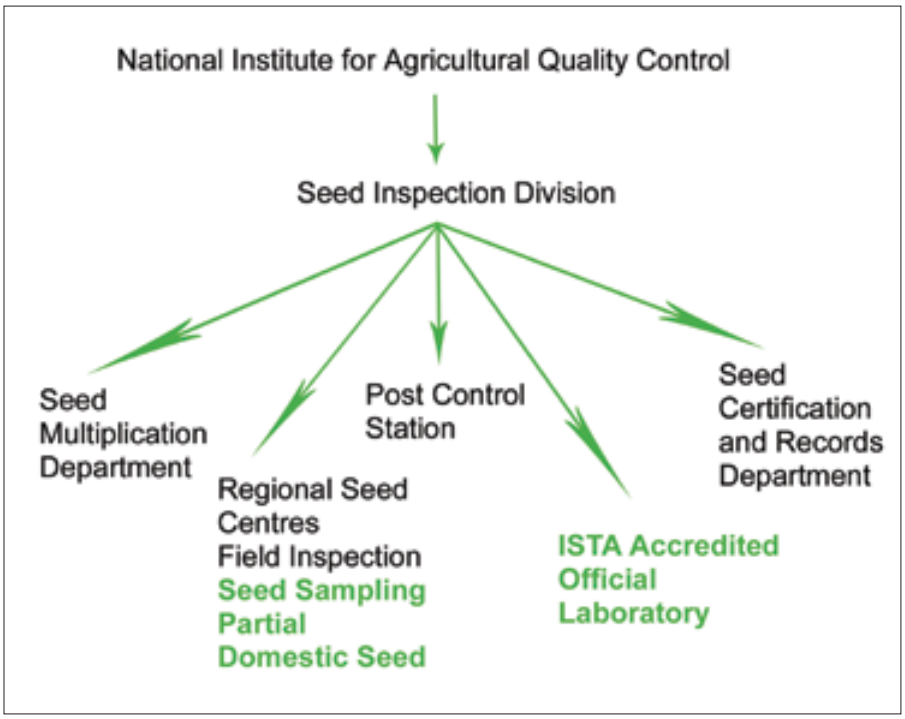


Figure 3. The position of the seed testing and sampling as part of the Seed Division

On page 7 you will find more information on the 27th ISTA Congress to be held in Hungary, and the Call for Papers for the Seed Symposium.

Or visit our website at www.seedtest.org

ISTA President, Prof. Dr. Norbert Leist

60th Birthday Celebration

By Adolf Martin Steiner, ISTA Member

Prof. Dr. Norbert Leist, Director of Biology and Head of the Division of Seed Testing and Applied Botany at the State Agricultural Experimental and Research Station Augustenberg (LUFA), Karlsruhe, Germany, celebrated his 60th birthday on 31st of December 2002. Born and grown up in Bruchsal, a town near Karlsruhe, he graduated from secondary school of classical education and was awarded the Scheffel Prize for excellence in the German language in 1962. While in the military service as a 2nd Lieutenant of the reserve he became familiar with such areas as planning, organization and leadership and, as a paratrooper, with decision making and objective-oriented behaviour. Already in his younger years he was fascinated by the miracles of nature. Hence, he began studying biology, chemistry and physics at the University of Heidelberg. After the state examination in these fields in 1968 he prepared a dissertation on "The ontogenesis of the reticular venation of fern leaves" under the guidance of Prof. Dr. W. Hagemann. In 1972 a doctor's degree of natural sciences was conferred on him. Supported by the German Research Foundation (DFG) a one-year stay in Columbia for field research on the ecology of tropical ferns and wood ecosystems followed. After terms as research assistant at the Universities of Karlsruhe and Heidelberg in the following years, he joined the staff of the LUFA Augustenberg in 1977 and, in 1992, became the successor of Dr. B. Schmidt as Head of the Division.

Prof. Leist started his career with a high degree of competence, an admirable endurance and prudent sense of enforcement. He developed his division to the most modern, internationally competitive German seed testing laboratory. With him seed matters in the State of Baden-Württemberg are in good hands. In addition, he enhanced counselling and research activities. Main areas of research are quality seed production in maize, wild oat, oat hybrids and fatuoids population biology in seed production, cereal seed health assessment, and studies on toxicity effects of seed dressings. Furthermore, there are numerous studies on the improvement of the methodology in tetrazolium testing and for the application of this test to new species. More recently, a great deal of activity was devoted to verification of cultivar by storage protein markers developing UTLIEF techniques, in particular for testing hybrid purity in seeds of maize and vegetable species. These studies were extended now by using molecular DNA techniques to test for adventitious GM seeds in conventional seed lots. The Association of the German Agricultural Experimental and Research Stations (VDLUFA) honoured his research activities already in 1981 with the rarely awarded Friedrich-Nobbe Prize. He also continued his comprehensive teaching program at the University of Karlsruhe to help fascinate students with applied botany and seed science and technology. There, his intellectual curiosity about nature together with his ingenuous ability to marvel at wonders hitherto attracted 3 post-graduate students and another 37 graduates preparing their theses under his guidance. For these outstanding achievements he was awarded an honorary professorship in 1992. Besides, he organised and was leader of many national and ISTA workshops on seed sampling, tetrazolium testing, cultivar purity testing by protein electrophoresis and ISTA quality management. Moreover, acknowledging also his singular merits in vocational training, the Chamber of Commerce of the Upper Rhine Valley bestowed on him the Carl-Friedrich-Nebenius Medal.

Active participation in associations accompanies Prof. Leist`s daily work in a true-to-life manner. Since 1978 he has been a Member of the Board of the Seeds and Seed Science Section of the VDLUFA and, in 1990, was elected Vice Chairman. As of 1980 he has been a Member of several ISTA Technical Committees: Purity, Forest Tree and Shrub Seeds, Variety, Tetrazolium (Chairman 1990 to 2000) and Rules Committees. In 1995 he was elected Member of the ISTA Executive Committee and in 1998 became 1st Vice President of ISTA. Already in 2000 he took over the ISTA presidency when President Kevin Boyce parted before his regular three years were up. In 2001 Prof. Leist was confirmed by the ISTA General Assembly in Angers, France, as ISTA President for the period 2001 to 2004. In the now 79 years of ISTA history, he is the first German ever to be elected for that post. Besides his strong involvement in the VDLUFA and in particular the ISTA, he is a member and active in several responsible positions in numerous other scientific associations in the fields of crop science, plant breeding, floristics, limnology and his beloved hobby arachnology. Also he is member of several state and federal committees of experts in these fields.

It is not possible to describe here the surprisingly broad interests and activities of Prof. Leist in more detail. His multi-faceted natural gifts, his widespread and profound scientific and practical knowledge and skills, his open-mindedness and readiness to listen, his politeness and well-balanced temper, his co-operative attitude and creative power combined with fairness and, above all, constantly positive attitude towards life in general are characteristics for which he earned national and international acceptance and high regard.

May Prof. Leist, in the years to come, be granted the opportunity to harvest rich crops while borne by his understanding family and supported by his loyal team of co-workers and well-tried colleagues. He loves hiking in the mountains and roaming tropical forests, diving into the mysterious underwater world of old father Rhein as well as into the magic of coral reefs. May all this give him the necessary "vigour". For seed science and technology as well as seed industry and trade, the VDLUFA and the ISTA, and his colleagues: we all need him. Happy Birthday and many happy returns.

Centenary of Seed Testing in Canada

Canadian Food Inspection Agency's Seed Laboratories Celebrate 100 Years of Seed Testing

By Alexander B. Ednie, Head of Seeds Section, Ottawa Laboratory (Carling) and ISTA Member
Janine Maruschak, Acting Head of Seed Science and Technology Section, Saskatoon Laboratory and ISTA Member

On December 5th, 2002, the Laboratory Directorate of the Canadian Food Inspection Agency (CFIA) hosted a reception to celebrate the hundredth anniversary of the establishment of the Ottawa Seed Laboratory and the federal seed testing program. The establishment of the laboratory marked the start of the seed program in Canada, which was aimed at improving the quality of domestic and exported seed, as well as protecting the virgin agricultural land that was being opened up for settlement in the Western part of Canada from the introduction of foreign weeds. For more of the history, see the Ottawa Seed Laboratory's profile (CADL04) on the ISTA web page section for member laboratory profiles.

The reception was attended by the staff of the Ottawa Laboratory as well as former staff that had been involved with the laboratory and had contributed to the development of the science of seed testing in Canada. Guests included staff from the Agency's Programs Branch, the Laboratory Directorate and colleagues from Agriculture and Agri-Food Canada's Research Branch that had collaborated with the seed laboratory over the years.

The highlight of the reception was the presentation by Mr. Richard Fadden, the President of the Canadian Food Inspection Agency of a commemorative plaque from Canada's Minister of Agriculture. In his presentation speech, the President praised the dedication of CFIA staff, current and retired, on their long-standing contribution to the public good and its strong history of international participation and co-operation.

We were pleased to have with us a number of special guests who brought greetings and congratulatory remarks for the occasion. These included Dr. Michael Muschick, Secretary General of ISTA, representatives from the Canadian Seed Growers Association, the Canadian Seed Trade Association, the Canadian Seed Institute and the Commercial Seed Analysts Association of Canada. Commemorative plaques were also received from the Association of Official Seed Analysts (the Ottawa laboratory has been a member since 1908) and the Society of Commercial Seed Technologists.

Displays of seed testing equipment from the early 1900s, archival photos, documents and pictures drew many exclamations and comments as guests revisited the past. Commemorative pins incorporating a design by Ken Allison, seed biologist at the Ottawa lab, were given to staff and guests to mark the occasion. As part of the centenary celebration, Sandy Ednie presented Mr. Fadden with a beautiful water color illustration of seeds that was one of many done by W.H. Wright in the 1930s and 1940s to help train seed analysts in seed identification. The illustration now hangs in the lobby of the CFIA Headquarters building.

The celebration held in Saskatoon commemorating 100 years of seed testing in Canada was a huge success. There were over 80 people who attended the December 17th event, including guest speakers from the Agriculture and Agri-Food Research Station, Commercial Seed Analyst Asscociation of Canada, Saskatchewan Agriculture Minister's Office, AgWest Biotech and Saskatchewan Seed Growers Association. Joanne Hinke, Purity Lab Supervisor and Janine Maruschak, Acting Head of the Western Seed Laboratory gave a presentation on the history and current status of seed testing in Canada. Many of the speakers focused on the close working relationships amongst the seed testing groups and those that rely on their services within Canada. There was praise from researchers, associations and clients that have worked closely with the Saskatoon Seed Lab and there was recognition of the exciting times in seed testing to come. For the many retired staff on hand it was a chance to greet friends and former colleagues. The Federal Agriculture Minister, Lyle Vanclief's commemorative plaque was presented by Liz Singh, Associate Executive Director, Laboratories Directorate to the seed lab staff. Guests and media took the opportunity to tour the new seed laboratory, which is located in Innovation Place, a research park adjacent to the University of Saskatchewan campus. The Saskatoon reception also provided an opportunity for the Western Seed lab to announce its new name - the Seed Science and Technology Section of the Saskatoon Laboratory. Print and TV Reporters were on hand to cover the event and take video footage of the laboratory and analysts, resulting in an article in the local newspaper and a segment on the local news and on a weekend agricultural television program.

Several articles have appeared in the popular agricultural press covering the centenary and the role of the seed laboratories in Canada's agricultural system. ■

India Applauds Danish Aid

University of Mysore, Mysore, India

Assistance provided by Danida (Ministry of Foreign Affairs, Denmark) to India in the field of Agriculture was recently acknowledged by the University of Mysore, India, when Director Dr. S.B. Mathur of the Danish Government Institute of Seed Pathology for Developing Countries (DGISP) was awarded Prof. K.M. Safeeulla Gold Medal on December 2, 2002. The gold medal was instituted for the first time by the university in memory of K.M. Safeeulla's significant contributions in the field of plant diseases, especially for internationally known research on downy mildew diseases of millets.

The medal was awarded for Dr. Mathur's expertise in seed-borne fungal diseases of tropical and subtropical crops as well as his knowledge of technological developments in seed health that that have inspired a generation of scientists all over the world. Dr. Mathur's contribution to increased food production through the use of improved seed that have been internationally acknowledged. Considering his achievements and contributions he was awarded with the prestigious 'FIS World Seed Prize' in 1992. Dr. Mathur is responsible for establishing a seed pathology research and training centre at the University of Mysore.

The Danish Institute of Seed Pathology in Denmark that have been Dr. Mathur's brainchild and he along with Dr. Paul Neergaard that have been responsible for founding the Institute. The Institute is located on the campus of the Royal Veterinary and Agricultural University of Denmark (KVL). The institute that have trained 75 agricultural scientists and technologists from all parts of India. The trainees came to Denmark from universities, research institutes, seed production and seed certification agencies. The main objective of the training that have been to improve the health of seed, with ultimate goal of increasing and improving food production. Professional and technical support received by the University of Mysore for over 25 years was highlighted by Dr. Shekar Shetty, Professor of Applied Botany at the medal presentation ceremony.

Danida is presently upgrading research and training facilities at Mysore and it is anticipated that the University of Mysore will eventually become a Seed Pathology Centre in Asia where scientists and technicians from different countries can be trained and they should then fight seed transmitted diseases which negatively effect food production.

Assistance to developing countries in the field of seed pathology and seed health by Denmark is considered by Indian Scientists as a success story of Danida.

Dr. V. Prakash, Director, Central Food Technology Research Institute, Mysore presenting the Professor K.M. Safeeulla Gold Medal and citation to Dr. S.B. Mathur (right) Director, Danish Government Institute of Seed Pathology for developing countries, Denmark.

Press Release

Minutes of the ISTA Extraordinary Meeting 2002

Cited and Published
By the ISTA Secretariat

This document summarizes and concludes the discussions and decisions of the ISTA Extraordinary Meeting 2002 in Santa Cruz, Bolivia, July 5 and 6.

As minutes of the subsequent meeting of the ISTA membership, this transcript proceeds the Post-Congress Proceedings of the 26th ISTA Congress 2001, Angers, France, published as Volume 29, Supplement 3 of Seed Science and Technology (SST).

These minutes contain a complete list of all participants of the meeting.

Available as of April 2003 from the ISTA Secretariat.

Price: **CHF 70.00** (approx. US$ / EUR 49.00)

Note: This publication will be delivered as a free service to all ISTA Members, Designated Authorities as well as to the participants of the ISTA Extraordinary Meeting 2002. It will also be distributed to all participants of the upcoming Extraordinary Meeting 2003 to be held in Zurich, Glattbrugg, Switzerland *(see more details on upcoming ISTA meetings from page 3 to page 7).*

International Seed Testing Association (ISTA)

ISTA Membership

Participate in the global network
Free copies of all new publications
Access to Technical Committees
Quality Assurance programme
Professional advise
Membership Certificates
Participation in ISTA Workshops, Seminars and other activities
Participation in the Proficiency Test Programme

All interested persons are invited to forward the attached request form to the ISTA Secretariat, PO Box 308, 8303 Bassersdorf, CH-Switzerland, phone +41 1 838 6000 or fax +41 1 838 6001, E-mail ista.office@ista.ch to receive a membership information package.

REQUEST FORM

☑ Yes, please send me more information on how to become an ISTA Member.

Contact Person .....

Organisation .....

Address .....

.....

City .....

Postal Code .....

Country .....

Phone .....

Fax .....

Email .....

New ISTA Members

JP-JAPAN Welcome!
JPML06 / JPPM06
Quality Assurance Department, Takii & Co Ltd, 180 Umekoji, Inokuma, Shimogyo-KUC.
P.O. Box 7, Kyoto 600-8686
Phone: +81 75 365 0123 Fax: +81 75 3650110
E-mail: mkomaba@takii.co.jp
Personal member: Mr. Masahiko Komaba

KE-KENYA
KEDM01 (additional member of KEDL01)
Mrs. Gladys. N. Maina, Kenya Plant Health Inspectorate Service (KEPHIS) Assistant Director, QC Service, Waiyaki Way, P.O. Box 49592, Nairobi
Phone: +254 2 440087 Fax: +254 2 448940
E-mail: kephis@nbnet.co.ke

Membership cancellation

AR-ARGENTINA
ARDM01
Ing. Agr. Adelaida Harries de Crespo
Presidencia Instituto Nacional de Semillas, Paseo Colón 922, 3° Piso, 1063 Buenos Aires

CL-CHILE
CLDM01
Dr. Ricardo Romero Alpe (additional member of CLDL01)
Corporación Nacional Forestal Special Advisor to the Executive Director
P.O.Box 10346, Santiago

TW-Separate Customs Territory of Taiwan, Penghu, Kinmen and Matsu
TWDM01
Mr. Chia-Chi Chen
Seed Testing Laboratory, Council of Agriculture, 37 Nanhai Road, Taipei 100-14

Mutations

AU-AUSTRALIA Welcome!
AUDM02
New personal member : **Dr. Kevin G. Boyce**
Seed Technology Institute, Australia Pty Ltd, P.O. Box 410, Blackwood SA 5051
Phone: +61 8 8278 3356 Fax: +61 8 82783356
E-mail: kgboyce@chariot.net.au
(formerly Mr. Colin Beavis)

DK-DENMARK
DKPM07
New personal member : **Ms. Dorrit Andersen**
L. Dæhnfeldt A/S, Germination Laboratory, Breeding Station Danefeld, Odensevej 82
5290 Marslev
Phone: +45 63 17 5506 Fax: +45 63 17 5505
E-mail: d.andersen@daehnfeldt.com
(formerly Mr. Finn V. Povlsen)

GR-GREECE
GRDM01
New personal member : **Ms. Eftychia Andritsou**
Seed Testing Station of Athens, Antheon 2, Marousi, 151 23 Athens
Phone: +30 10 6830471 Fax: +30 10 6830917
E-mail: alexef@aias.gr
(formerly Mr. Athanasios Kouis)

KE-KENYA
KEDM01
New personal member : **Dr. Joseph Oloo Ahenda** Kenya Plant Health Inspectorate Service, Nakuru Regional Office,
P.O. Box 1679, Nakuru
Phone: +254 37 850106 Fax: +254 37 851268
E-mail: kephissq@nbnet.co.ke
(formerly Mr. Jeremiah K. B. Kiplagat)

KR-KOREA, REPUBLIC OF
KRDM01
New personal member : **Mr. Jin-kug Kim**
Central Seed Testing Laboratory, Inspection Division, Experiment Research Institute of NAQS, 560, 3-Ga, Dangsan-Dong, Youngdeungpo-Gu, Seoul
Phone: +82 2 2165 6041 Fax: +82 2 21656005
E-mail: kimjk@naqs.go.kr
(formerly Mr. Kyu-won Choi)

RU-RUSSIAN FEDERATION Welcome!
RUDM01
New personal member : **Mr. Viktor Trusov**
The State Seed Inspection of the Ministry of Agriculture of Russia, Orlikov pereulok 1/11
107139 Moscow
Phone: +7 095 2073883 Fax: +7 095 2073883
(formerly Mrs. Tatyana Kokina)

RUDM02
New personal member : **Ms. Galina Filipenko**
Plant Germoplasm Conservation Laboratory, N.I. Vavilov, Institute of Plant Industry 42, Bolshaya Morskaya Street, 190000 St. Petersburg
Phone: +7 812 3147714 Fax: +7 812 3118728
E-mail: g.filipenko@vir.nw.ru
(formerly Dr. V. A. Zaitsev)

Seed Pathology at the 8th International Congress of Plant Pathology

Christchurch, Canterbury, New Zealand, February 2 - 7, 2003

By John Hampton,
ISTA Executive Committee Member-at-large

Christchurch, New Zealand was the venue for the 8th International Congress of Plant Pathology (ICPP 2003) which was held during the week of 2 - 7 February 2003. Nearly 1300 people from 72 countries participated, enjoying the scientific information transfer, the chance to meet fellow plant pathologists, and the famous Kiwi hospitality. Under the theme of "Solving problems in the real world", three plenary sessions, five keynote sessions, 32 concurrent sessions and two poster sessions provided delegates with the opportunity to immerse themselves in all areas of plant pathology, including seed pathology.

Concurrent Session 22 (Seed Pathology) was organised by ICPP 2003 Programme Committee member Professor John Hampton, with assistance from ISTA PDC chairperson and deputy chairperson Jim Sheppard and Valerie Cockerell. This provided an opportunity to showcase the current work of the PDC, particularly in seed health method validation and quality assurance and proficiency testing. The session was chaired by PDC member Dr Guro Brodel from Norway.

Jim Sheppard (Canada) briefly explained ISTA's purpose to the 160 strong audience, before presenting the ISTA Seed Health Method Validation Programme. Jim demonstrated the steps required before a final product (a validated seed health test method) can be included in the ISTA Rules. He also outlined the progress made to date, and some of the problems encountered. His presentation was followed by one from Valerie Cockerell (Scotland) who explained the ISTA accreditation process and discussed how seed health testing could be brought into the ISTA proficiency testing programme. This will be no easy task, and there are a number of issues still to be resolved, including the availability of suitable seed lots, the application of performance standards, and the difficulty of annually assessing individual labs which may not all be performing the same seed health tests.

The third speaker was PDC member Dr. Steve Roberts (England), whose topic was the use of reference materials in seed health method validation and proficiency testing. Steve made the point that variability in the test material (i.e. infected seed lots) is a major limitation to the success of comparative testing and the interpretation of their results. He suggested that the use of Standardised Reference Materials (RM) containing a stable quantity of the pathogen was one way to overcome the problem. While initially costly to produce, RMs will be cost effective in the long term, and an EU supported project has already resulted in RMs for four seed transmitted bacterial pathogens. Inter-lab studies using these RMs have demonstrated their value for accuracy and precision of seed health assays.

The session concluded with a demonstration by former PDC Chairperson Dr. Kees Langerak (The Netherlands) of how a validated seed health method can be used in practice, using *Alternaria radicina* in organic carrot seed production. He described laboratory and field studies which determined to what extent seed infection can contribute to seedling death, storage black rot and seed to seed transfer of the pathogen, the objective being to determine pathogen thresholds for the validated seed health test method.

As the session organiser, chairperson and speakers were all ISTA members, the audience left having broadened their knowledge of ISTA and the work of the PDC. In addition, the ISTA information available to the audience (which included the Publications Catalogue and News Bulletin) disappeared within minutes!

There was also a Seed Pathology section in Poster Session Two in which 21 posters by authors from 10 countries were displayed. Twelve of these authors then had the opportunity to make a brief (5 minute) oral presentation during the poster discussion session. Jim Sheppard chaired this session, during which PDC Member Rivka Hadas (Israel) spoke about her work on the development of a PCR detection method for *Xanthomonas campestris* pv. *campestris* in brassica seeds.

Seed pathology received a much higher profile at ICPP 2003 than it has at previous International Plant Pathology Congresses. Jim Sheppard made the point several times that ISTA Membership is not a requirement for submission of a seed health testing method to ISTA for consideration for validation. The opportunity to present ISTA and its PDC to a wider audience will encourage such submissions, as seed pathologists not already working within ISTA are now aware of this work.

OECD Scheme for the Control of Forest Reproductive Material Moving in International Trade

Paris, France, October 1 - 4, 2002

By Dale Simpson, ISTA Forest Tree & Shrub Seed Committee Member

The biennial meeting was attended by representatives from 19 Scheme member countries and 4 other organizations including ISTA. Membership in the Scheme is voluntary and open to OECD Member countries as well as all members of the United Nations.

The prime focus of this meeting was to seek a means of approving a new Scheme. The current Scheme, which was adopted in 1974, was viewed as having become somewhat dated so work began on developing a new Scheme in the early 1990's. In 1996 the Scheme was presented for approval by member countries however, there was a reservation regarding the inclusion of Genetically Modified Organisms (GMO) which resulted in the inability for the Scheme to be adopted by OECD. Several attempts have been made to revise the Scheme but the proposed changes were not accepted by all member countries. Another consideration was the European Union's (EU) new Directive on the marketing of forest reproductive material scheduled to come into force January 1, 2003. This Directive, which was developed in parallel with the new Scheme, contains references to GMO. The EU recognizes "equivalence" between their directive and the OECD Scheme so it is important that the new Scheme be approved to provide harmony when reproductive material is traded between EU and non-EU countries. Following lengthy, productive discussions a recommendation was made to form an expert group to revise those portions of the Scheme referring to GMO and submit the revised Scheme to member countries for their approval. The expert group planned to meet in December.

Additional agenda items covered at the meeting included a document summarizing, for each of the four categories, weights of forest seed certified, used, or traded by participating countries in the 2000-2001 time period. Total weight of seed certified for domestic use amounted to a record of 1 363 tonnes. An updated list of approved basic material was presented. It illustrated that the "Source-identified" category, the most basic level of genetic quality, was still very important for a number of countries with a total area of 13 million hectares of forest from which seed is collected. The "Selected" category consisted of 515 000 ha. "Untested seed orchards" comprised 2 600 ha while the "Tested" category, containing stands and seed orchards, had 3 400 ha. Clones and clonal mixtures were also part of this category for a number of countries. The Slovakian delegate provided an interesting presentation about seed management and certification activities in Slovakia which are compatible with the OECD Scheme. Presentations were also made by OECD staff on consensus documents developed for a number of forest tree species useful for people dealing with regulatory assessment of genetically modified material and the creation of a web page dedicated to the forest seed Scheme. Statements were also presented by representatives from FAO, CPFUE (Committee for Forest Nurseries of the EU), ISF, and ISTA. The delegates were hosted by Switzerland for a one day visit to the area adjacent to Lausanne to visit stands of Douglas fir and Norway spruce, field trials of foreign tree species, and several other stops.

CSSA Symposium on Worldwide Seed Health Test Standardization Systems

Indianapolis, Indiana, USA, November 2002

By Steve Roberts,
ISTA Plant Disease Committee Member

The CSSA Symposium on Worldwide Seed Health Test Standardization Systems was held on 13 November 2003. This symposium was part of the much larger joint annual meeting of the American Society of Agronomy (ASA), the Crop Science Society of America (CSSA), and the Soil Science Society of America (SSSA). The meeting attracts some 4,000 participants mainly from North America, but also from around the world, representing academia, government and private industry, and including a large contingent of undergraduate and graduate students. The scale of the meeting was quite awesome with many concurrent sessions/symposia and a cavernous exhibition area for many hundreds posters and trade displays. The symposium started with presentations by Denis McGee from the Seed Science Centre of Iowa State University, on the USA National Seed Health System and Rudy Scheffer, the chairman of ISHI-Vegetables, on the industry-backed International Seed Health Initiative. Steven Roberts, leader of the ISTA-PDC Bacteriology Working Group, then gave a presentation on the ISTA-PDC Method Validation Programme covering such topics as: the need for method validation; the development of the ISTA programme; the steps involved method validation and the progress to date. Although the audience was disappointingly small for such a large meeting, this did mean that those present were all the more enthusiastic leading to a lively discussion after the formal presentations.

13 Feb 2003

ISF Press Release

2003 ISF Congress

The 2003 ISF Congress will take place in Bangalore, India on 9-11 June 2003.

Bangalore will host the first congress of the International Seed Federation (ISF) formed from the historic merger of FIS and ASSINSEL, two associations established in 1924 and 1938, respectively. With participants from over 65 countrties expected at the biggest international gathering of seedsmen and seedswomen from all the world over, the congress provides a forum for:

- discussion on the main issues of interest or concern to the seed industry at a global level
- meeting colleagues for scientific, technical or commercial purposes
- learning more about the Indian seed industry and market

India is credited with a strong seed industry and is one of the largest unified national markets in the world. It is endowed with strong skills and state of the art technology at all levels, from research to seed production.

The ISF Seed Treatment and Environment Committee (STEC) has organised a Seed Treatment Conference also to be held in Bangalore, after the congress on 12 and 13 June 2003.

The full information package onthe congress is available on-line at www.worldseed2003.com.

For practical information contact the congress organizers at:

Conferences & Incentives
Management (I) Pvt. Ltd.
C1/D, 1st Floor
Green Park Extn
New Delhi 110 016
India
Tel: +91 (11) 2653 6075
Fax: +91 (11) 2653 6086
Email: cimindia@vsnl.com

Main items on the agenda

- Essential derived varieties and in particular principles for a code of conduct in Lettuce
- Arbitration procedures for dispute settlement during trade in seeds for sowing and for the management of intellectual property
- Genetically modified varieties:
  - Impact on the seed industry
  - Position of ISF on Adventitious Presence and GMO testing
- Presentation of the Indian seed industry and seed regulations
- FAO MTA on access to genetic resources

More information can be found in the detailed program

For more information, please contact the ISF Secretariat:

Chemin du Reposoir 7, 1260 Nyon, Switzerland
Tel: +41 22 365 44 20, Fax: +41 22 365 44 21
E-mail: isf@worldseed.org, Web: www.worldseed.org

CALENDAR

2003

May

12-16 ISTA Flower Seed Testing Workshop
(Budapest, Hungary)

14-16 ISTA Seed Vigour Testing Workshop
(Parndorf, Austria)

June

09-11 ISF Congress
(Bangalore, India)

11-13 ISTA Purity Workshop
(Seattle, United States)

July

30-03 ISTA Extraordinary Meeting 2003
(Zurich, Switzerland)

07-11 OECD Annual Meeting of the Seed Schemes
(Paris, France)

October

20-25 ISTA Forest Tree and Shrub Seed Workshop
(Prague, Czech Republic)

November

03-07 ISTA Moisture Workshop
(Lyngby, Denmark)

2004

May

13-24 ISTA 27th International Seed Testing Congress
(Budapest, Hungary)

24-26 ISF Congress
(Berlin, Germany)

International Seed Testing Association (ISTA)

ISTA Secretariat, Zürichstrasse 50, PO Box 308, 8303 Bassersdorf, CH - Switzerland
Phone: +41 1 838 6000, Fax: +41 1 838 6001, E-mail: ista.office@ista.ch

www.seedtest.org