

# Seed Testing INTERNATIONAL

ISTA News Bulletin No. 128 October 2004



Read more about the  
AVRDC/ARC/APSA/ISTA  
Seed Testing Workshop

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# Seed Testing INTERNATIONAL



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# Editorial

By Michael Muschick,  
ISTA Secretary General

Dear Reader,

In this first issue after the successful completion of the 27<sup>th</sup> ISTA Congress 2004 in Budapest, Hungary this May, I would like to thank all participants and the local organising team for their contribution, with particular emphasis on our Technical Committee representatives and other Members for their outrageous work making this meeting a first-class event.

Some decisions taken in Budapest have a direct effect on this magazine: an other person than it used to be for the last 6 issues has written the President's Address on page 2, and I would like to welcome Ir. Pieter Oosterveld in his new position as President of our Association.

We do not only have a new President since the Congress, but we have a whole new Executive Committee for the coming three years. Before I welcome the new members however, I would like to thank those members that stepped back in Budapest; first and most of all Prof. Dr. Norbert Leist, who was our President for the last three and a half years and with whom I had the pleasure to work very closely and intensively with during this time. But also the Members-at-large which resigned from the Executive Committee will be missed, both work wise and as persons, and we owe them a lot of gratitude: Mr. Doug Ashton, Mr. Ronald Don, Ing. Monica I. Moreno, Dr. Jeffrey Luhanga and Dr. Chulhathep Pongsroydech. All the same, I would like to heartily welcome and congratulate our newly elected Members of the Executive Committee, some of them re-elected and therefore warmly welcome again for a next period and thank you for keeping up your support and for your commitment to serve the Association for the next triennium: Dr. Joseph O. Ahenda (newly elected), Mr. José M. Chávez Bravo (newly elected), Dr. Katalin Ertsey (re-elected and newly elected as 1<sup>st</sup> Vice-President), Prof. Dr. John Hampton (re-elected), Dr. Steve Jones (newly elected), Dr. Joël Léchappé (re-elected), Mrs. Susan Maxon (newly elected), Prof. Dr. Silmar T. Peske (new 2<sup>nd</sup> Vice-President), Mrs. Grethe Tarp (re-elected), Dr. Rita Zecchinelli (newly elected), and last but not least, our new President, Ir. Pieter Oosterveld.

By reading this issue of *Seed Testing International*, you will be sure to find more reports from the Congress, completed by the Minutes of the Ordinary Meeting held May 20 and 21, which have been published by the Secretariat and are available online for download, together with a number of power point presentations offered during the meeting. Moreover a collection of photographs taken during the Congress is available on the website - if

you would like to refresh your impressions gathered from the meeting or to get some impressions if you maybe did not have the chance to enjoy the meeting 'live' in Budapest.

Yet, the memories from this last ISTA Meeting are still fresh, we are already announcing the next one: the ISTA Ordinary Meeting 2005 to be held April 25 - 28 in Bangkok, Thailand, upon the obliging invitation from the Department of Agricultural Extension (DOAE). Please read about the programme, registration and the fascinating city of Bangkok in the announcement published some pages further back this issue.

Talking about meetings, I should not miss to mention the first World Conference on Organic Seed which was held successfully in Rome in July and to which ISTA contributed as a partner organisation. A note on this conference can be found under the heading 'Meetings' as well as a more general contribution to the subject under 'Issues of common technical interest'.

Another frequently seen subject under this theme is of course the one of GMO testing. In this section we are privileged to give you an insight in the ISTA GMO Proficiency Test by a contribution from two of our experts in this field. Plus, as an other highlight in the same area, ISTA is announcing its 4<sup>th</sup> ISTA GMO Proficiency Test Round, starting this month. Please take more details on this test round and how to participate from the announcement on page 48.

During the past six months, ISTA has been active in many regions, geographically as well as technically: please read the reports of the several workshops held, questionnaires conducted and general insights to the work of our Technical Committees.

On a personal note, but still in relation to Technical Committees, I would like to congratulate our Head of Technical Committee Administration, Bettina Kahlert, who has become a proud mother of a son in early August. All staff at the Secretariat wishes Bettina and her little family all the best and are looking forward to welcome her back to work by the end of the year.

Now with the various subjects covered in this issue, I hope you will find it an inspiring and contributing magazine.

All the same, please remember that you are always welcome to send in comments, suggestions or any interesting contribution.

Yours sincerely,  
Michael Muschick



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The 1<sup>st</sup> Announcement for the ISTA Ordinary Meeting 2005 is now available

Registration forms have been included in this issue, while online registration has been available since October 1<sup>st</sup>, 2004

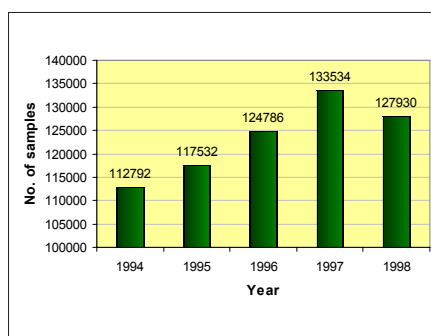
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## Healthy seeds, the basis for sustainable farming



We continue the series of papers which were presented at the 4<sup>th</sup> ISTA-PDC Seed Health Symposium, held in Wageningen, Netherlands, in 2002

**Page 14**



Read about the Comprehensive Worldwide Questionnaire on Flower Seed Testing

**Page 23**



The 4<sup>th</sup> ISTA/FAO Workshop on GMO detection was held in Slovenia.

Read about GMO testing in Eastern European countries and specifically Slovenia

**Page 30**



Steven Groot reports back on the First World Conference on Organic Seeds held in Rome in July of this year

**Page 46**

# President's Report

By Pieter Oosterveld, ISTA President

Our colleagues in Hungary hosted the 27<sup>th</sup> ISTA Congress very successfully. Many ISTA members, many of them accompanied by their partners, visited the beautiful city for the first time and enjoyed the very nice atmosphere.

The programme started with the Purity workshop. It took the organisers just a few months to set up an attractive programme. This workshop was attended by 34 participants.

The Technical Committees presented their work during the open committee meetings. Many results of the work over the past three years were presented to an interested audience. It was clearly shown, that since ISTA has annual meetings, the work of the Technical Committees is more productive. Since ISTA has reduced the number of days for the congress, we can not avoid that committees meet at the same time in parallel sessions. Some participants made their complaints on that.

The Symposium was successful, as well. The conference room was impressive and provided a good ambience for the scientists to present the results of their research. The Seed Symposium was attended by more than 400 participants from all six continents. For more information, see Alison Powell's report of the Symposium on page 4.

At the Ordinary Meeting we had to discuss a number of important items, the inclusion of a GMO chapter into the ISTA Rules, the accreditation of seed company laboratories and the voting rights.

The GMO Task Force had a very busy congress. I think that this Task Force met for over 15 hours during the days before the Ordinary Meeting. Representatives of governments, seed industry, and international organisations discussed about the methods, the results of the proficiency tests and ISTA's policy. It was agreed, that ISTA has chosen the right way by following the performance based approach. So, ISTA is not going to include methods in the rules, but will describe the criteria for GMO testing and the performance. It is expected, that final decisions will be taken at the Ordinary Meeting in Bangkok, 2005.

The discussion on the accreditation subject indicated clearly, that ISTA welcomes seed

company laboratories in the accreditation programme. It was agreed, that accreditation is a technical process and laboratories, when accredited, are considered to be technically equivalent. It was decided that laboratories, when accredited, are automatically authorised to issue ISTA Certificates.

The discussion on the voting rights in ISTA was a very principle one. After consultative discussions in the previous years, the Executive Committee of ISTA had prepared a proposal allowing all laboratories to vote on technical matters. With this proposal, the Executive Committee aimed at increasing the interest of seed companies to participate in the work of the Association. Many delegates indicated their concerns about ISTA's independent status in case the proposal would be accepted. The Executive Committee explained that the change of the voting rights, as proposed, only included the voting on technical matters. At the end of the day the meeting voted against the proposal.

Recently Doug Ashton, Sandy Ednie and Jim Sheppard decided to retire from their work in ISTA. These three Canadians have contributed during a long period a lot to the aims of the Association. Sandy served the Association as President and Chairman of a number of Committees. Doug was very active and productive as Chairman and Member of the Germination Committee and the Proficiency Test Committee. Furthermore he was member of the Executive Committee for nine years. Jim concentrated mainly on the Seed Health Committee. He was a very active Chairman and Member of this committee. Furthermore he used to be the ISTA webmaster and developed the ISTA website. The Executive Committee concluded that these three persons have done very important work for the Association and decided to honour them with the ISTA plaques.

After election, the newly elected members of the Executive Committee were installed. I like to thank Doug Ashton, Ronnie Don, Jeffrey Luhanga, Monica Moreno and Chulhathep Pongsroypech, who, for various reasons, did not continue their membership in the Executive Committee, for the work they have done.

As a good tradition, the participants were invited for a number of social events and



after the Congress a very interesting Post Congress tour was held.

We said farewell to Hungary, not before we said good bye to Professor Dr. Norbert Leist as outgoing President of the Association. Norbert started his presidency in December 2000. During his presidency, ISTA continued the policy of changes. Norbert has showed to be a President with a very high level of technical know-how and a clear view on the strategy of the Association. These two elements, combined with his personal interest in the people working for ISTA, made him a great President. We are very grateful to Norbert for continuing his chairmanship of the GMO Task Force. We thank Norbert for all the work he has done. We also thank Norbert's wife Alice and his family for their support.

On behalf of all the participants, I would like to thank the Hungarian government and all the colleagues in Hungary for their hospitality and the excellent organisation of the Congress 2004.

The newly elected Executive Committee held its first meeting in Budapest, straight after the Congress. The main goal of the meeting was to prepare the work for the years to come. We have installed a number of working groups that are going to elaborate ideas and prepare proposals for discussion at the meeting of the Executive Committee from 17 to 19 January 2005 in Switzerland.

As presented in Budapest, ISTA will pay special attention to the matter of tropical seeds. Grethe Tarp, who is prepared to chair this working group, has sent out a questionnaire. I have contacted FAO in order to explain the ideas of ISTA in relation of tropical seeds.

We agreed on further collaboration, e.g. by organising workshops.

Again in Budapest, ISTA and AOSA/SCST agreed on a new approach in relation to harmonisation of the rules. A working group has worked for many years on harmonisation. However, the number of differences is tremendous, so the work seems to be endless. It was agreed to focus the work only on the differences that really bother the international trade. ISF (International Seed Federation) and ASTA (American Seed Trade Association) were invited to indicate these limiting differences.

The Executive Committee of ISTA will discuss the continuity of the Association. The Congress in Budapest showed clearly that ISTA is a vivid association with very active membership. ISTA is respected and the work of ISTA is appreciated by governments, seed trade, international organisations and laboratories. Taking into consideration the results of the Budapest Congress, the Executive Committee will prepare proposals for the policy of the Association for the benefit of all stakeholders. We invite you to help us by sending your ideas and comments.

Finally, I would like to thank everybody for their work for ISTA and their support to the success of the Association.

**Your President,  
Pieter Oosterveld**

# 27<sup>th</sup> ISTA Congress 2004

## General Report

**By Sarah Meier, ISTA Marketing**

The 27<sup>th</sup> ISTA Congress 2004 was held in the beautiful city of Budapest, Hungary, from May 13 - 24. The Congress was well attended by more than 400 participants, representing all 6 continents, and was a resounding success, not only due to the number and diversification of the delegates, but also due to the excellent organisation by the local organisers, Dr. Károly Neszmélyi, Dr. Katalin Ertsey, Mrs. Zita Ripka, and the Conference Secretariat, the ISTA Secretariat, the Symposium Convenor, Dr. Alison Powell, and the ISTA Technical Committee Chairs.

The ISTA Technical Committee Meetings were held on May 13 - 14. These meetings were well attended by Technical Committee Members and observers. The Committees used this opportune moment to not only present activities and achievements, but to map out future directions.

Monday morning, May 17<sup>th</sup> saw the Opening Ceremony for the Seed Symposium, which continued for 3 days. Please read Dr. Alison Powell's report on the Seed Symposium on page 4 for more insight.

The ISTA Ordinary Meeting was held May 20-21. This meeting was a historical moment in ISTA's history, and many topics were discussed and Constitution changing votes were taken. For more details on these decisions, the Minutes of the Ordinary Meeting are available from the ISTA Secretariat (see page 22 for ordering details).

May 22 - 24 offered the opportunity to delegates for various post-congress tours.

The social events were most enjoyable to all. A Welcome Reception, organised in the mar-



vellous building of the Museum of the Hungarian Agriculture, was held on May 17<sup>th</sup>, and on May 19<sup>th</sup>, the Congress Dinner, on board of the 'Europa' boat cruising on the river Danube from where all were able to admire the evening lights of the capital city, and were treated to entertainment and a fireworks display.

The ISTA Secretariat would like to take this opportunity to thank all the delegates for their support of the Congress, many of whom travelled a great distance to participate and ensure that the Congress was the great success that it was.



# ISTA Seed Symposium

## Towards the Future in Seed Production, Evaluation & Improvement

By Alison A Powell, ISTA Seed Symposium Convenor 2004

University of Aberdeen, Department of Agriculture and Forestry, United Kingdom, e-mail: a.a.powell@abdn.ac.uk



It is difficult to think of a more appropriate combination of time and place for the 27<sup>th</sup> ISTA Congress and Seed Symposium than Budapest in 2004. This was the year that Hungary, along with other East European States, began to look forward to the future as part of European Union. Fittingly the Seed Symposium title and theme was 'Towards the Future in Seed Production, Evaluation and Improvement' and the venue was in a country with a long tradition of seed production. Hungary was well represented in the Symposium, which was attended by over 400 participants from all six continents.



The Symposium was led by three keynote speakers, who addressed three aspects of ISTA's work, namely links with the seed industry, education and training and the application of new test methods. The first keynote speaker, Zoltan Syposs from Syngenta, Hungary, considered the Future Development of the Seed Industry in Eastern Europe. Whilst many changes had already occurred in Hungary since 1989, he reviewed the new opportunities that would be open to all the new accession states following EU

membership. His presentation clearly showed that the seed industry in Eastern Europe is well positioned to make the most of these opportunities. In contrast the future was less clear in the second keynote presentation, the Development of Education and Training in Seed Science and Technology, given by Murray Hill from New Zealand Seed Technology Institute, Lincoln University. He noted that a decline in aid funding had reduced training efforts compared with the many successful programmes that had been available until the early 1990's. One approach he suggested to stimulate future training was for ISTA to take a proactive role in lobbying for resources to further support ISTA members activities in future international training programmes. The third and final keynote presentation, Molecular Methods and the Future of Seed Testing by Gerry Saddler (Scottish Agricultural Science Agency, UK) dealt with a topic that is very much part of the future and is developing rapidly. He reviewed the application of molecular methods in three testing areas, seed health testing, using a qualitative PCR assay as an examples, varietal identification using fingerprinting based on microsatellites and finally the detection and characterisation of GM contaminating events in seed lots.

The main purpose of the Seed Symposium is to provide a platform for the presentation and discussion of oral and poster papers contributed by seed scientists and technologists. Papers (oral and poster) were contributed from Europe (32% of papers), South America (25%), Asia (28%), the Middle East (8%) and Africa (4%), with fewer than 2% of papers coming from both North America and Australasia. This clearly illustrates where active seed scientists supportive of the Seed Symposium are located, the number of contributions from India, Iran and Brazil, where the next Congress will be held in 2007, being particularly prominent. These scientists came from diverse locations including universities, government departments and research institutes. Some of these were also ISTA laboratories, particularly so in the case of European laboratories.

The theme of the Symposium was followed in the offered papers within seven session topics, each supported by oral papers and two poster sessions. The papers covered many common agricultural and vegetable species, with, not unexpectedly, maize, soyabean and rice to the fore. In addition there were a number of papers on diverse and more unusual species. The emphasis of many of these papers was the future conservation of the species and the maintenance of genetic diversity in general. The few papers presented on flower seeds illustrated the problem areas, such as dormancy, that are a feature of these species, indicating an area of future work for seed scientists and technologists.

The first session topic **Application of Advanced Technologies** (Chairperson Enrico Noli) was clearly looking to the future. The oral papers described the use of a diverse range of techniques: a computerised key, NIRS and SSRs to recognise prohibited and restricted seeds, weed seeds and genetic diversity respectively. In contrast the greater proportion of the poster papers focussed on the application of DNA-based technologies such as PCR, AFLP and SSR's for purposes as varied as identifying stem lucerne nematode, genetic diversity in *Triticum dicoccum* and tolerance of soyabean to glyphosate. Posters focussing on identification of cultivars considered use of ELISA and SDS-PAGE, whilst image analysis and machine vision were applied to whole seeds and seedlings for different purposes. As a display of the potential techniques that are being developed, this session succeeded in alerting the Symposium participants to what could be the future in seed technology.

Seed production provides the foundation of quality seed and the increase in organic crop production and the production and evaluation of organically produced seeds provides new challenges. In the session **Organic and Conventional Seed Production** (Chairperson Jose de Barros Franca Neto) one oral paper gave an overview of issues and problems associated with organic seed production, while a second focussed specifi-

cally on the application of plant extracts for bunt control. The oral papers on conventional seed production dealt with different aspects of production of diverse species - wild flowers, castor, barley and sugar beet. Many of the poster papers (22%) focussed on aspects of seed drying and storage, while 15% considered hybrid seeds production. The remaining posters examined the effects of chemical treatments on the seed crop and aspects of production in a diverse range of crops.

By far the greatest number of oral and poster papers (50%) were submitted to Session 3 **Viability and Vigour: Evaluation and Impact** (Chairperson Joel Lechappe), providing an interesting combination of papers on the oldest (viability) and one of the newer (vigour) topics within seed testing. Papers were distributed evenly between these two topics, with approximately a third of the papers considering viability and vigour in the context of seed storage. The oral and poster papers on viability included studies on the influence of production conditions, stage of seed development, seed weight and germination conditions and surveys of germination behaviour. Many familiar crops were included such as wheat, sugar beet and cotton. However around 40% of papers were on seeds of wild plants, medicinal species, trees and shrubs, and a few on flower seeds, possibly suggesting more problems with future alternative crops and species introductions to increase genetic diversity. In general, the papers on vigour examined the application of ISTA validated and recognised vigour tests (such as accelerated ageing, conductivity, controlled deterioration) to a range of species including melon, coffee, lucerne and cotton. Only three papers described new proposed vigour tests. Papers on seed storage addressed the influence of storage container, fungicide treatment and fungal infection on storage life and comparisons were made of the storage life of hybrids and their parental lines.

Session 4, **Seed Systems in Developing and Emerging Economies** (Chairperson Grethe Tarp) included three oral papers, all focussing on rice seed production. The circumstances of each paper were contrasting. In Brazil, great improvements in yield were described following the elimination of red rice, the development of the seed sector in Vietnam as the country changes from a planned economy to one driven by market demand was reviewed and a simple on-farm approach to improve the rice seed quality, health and production was described in Bangladesh and Tanzania. Poster papers originated from Africa (3), Central and South

America (4) and from India, Croatia and the Czech Republic. These dealt with topics ranging from the development of a rural seed industry in Zambia, to breeding programmes for maize improvement in Mexico and an East European network to facilitate communication within the seed sector. The potential for the future development of the seed industry is considerable in the countries targeted within this session and hence the limited number of paper submitted was perhaps surprising. However, papers from these countries were also presented within other sessions.

**It is difficult to think of a more appropriate combination of time and place for the 27<sup>th</sup> ISTA Congress and Seed Symposium than Budapest in 2004. This was the year that Hungary, along with other East European States, began to look forward to the future as part of European Union.**

The session **Seed Lot Hygiene** (Chairperson Akos Mesterhazy) only included papers on seed borne disease, no papers were submitted on insects or weeds. Two oral papers focussed on traditional topics of seed pathology, namely factors affecting the incidence of infection and the impact of disease on emergence. Others looked more to the future, considering treatments of Brassica seeds against black rot, the impact of priming on seed borne pathogens and a comparison of seed health test results from conventional versus PCR based testing methods. Poster papers were largely divided into those focussing on the incidence and effects of pathogens and those examining seed treatments or other aspects of pathogen control. In the first group two papers took a new approach. One paper demonstrated the phytotoxic effects of the mycotoxins produced by *Fusarium* sp on the germination and ultrastructure of cowpeas, while the second provided microscopic evidence that polyphenolic compounds in coloured seed coats of Bambarra groundnut may suppress infection by fungi. Amongst the papers on seed treatment, over half considered alternatives to the use of chemicals for pathogen control such as thermotherapy, microwave treatment, biocontrol agents and biological products, perhaps another indication of the future in seed technology.

The oral papers in the session **Seed Improvement** (Chairperson Hugh

Pritchard) covered a wide range of seed treatments from priming in watermelon and rice, to scarification of tropical grass seed and the testing of insecticide effects on maize. Two further papers focussed in different ways on seed storage. One examined the use of different polymers to extend the storage life of onion seeds, while the other considered the use of rapid ageing techniques to evaluate the potential of seed treatments to prolong longevity. The poster papers covered similar topics to those in the oral session. These included 36% that dealt with germination stimulation and dormancy breaking, which, as one might expect included diverse and often unusual species such as turf grasses from Iran, *Protium heptaphylla* from Brazil and golden rain tree (*Koelreuteuria paniculata*) from Korea. Treatments examined included scarification, hydration, gibberellic acid, and smoke and fire. The application of well-recognised seed invigoration treatments (matricconditioning, PEG, hydro and drum priming and aerated hydration), mainly to common species, made up a further 27% of the posters. The remaining posters evaluated the ability of growth retardants, aqueous extracts of plant material and fungicides to enhance seed storage potential.

The final session in the Symposium **Physiological Basis of Seed Quality** (Chairperson Francoise Corbineau) was held in collaboration with the International Society for Seed Science and acknowledged the importance of the interaction between pure and applied scientists. However, as pure science becomes increasingly based in studies at a molecular level it is a major challenge to identify pure research that has a recognisable practical application. Seed deterioration and seed development each provided the physiological basis for two oral papers and a further paper examined the effect of post harvest methods on seed quality. Finally a novel approach to evaluating seed quality in limited stocks of conservation material was proposed, whereby a sample of only 100 seeds could be used to assess desiccation tolerance and germination prior to long term storage. A third of all poster papers also concentrated on seed storage, mostly on the physiological process of deterioration although one considered the application of QTL mapping to detect and localise genetic factors contributing to seed vigour and longevity. This was a good example of the potential application of molecular research to a practical problem in seed technology. It was also one of the many examples of papers in the Seed Symposium that drew the attention of participants to one of the directions that seed technology may take in the future. ■

# ISTA Ordinary Meeting 2005

Bangkok, Thailand  
April 25 - 28, 2005

Dear Colleagues,

The International Seed Testing Association (ISTA) takes pleasure in inviting you to participate in the ISTA Ordinary Meeting 2005, to be held from April 25-28, in Bangkok, Thailand.

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.

This meeting will also have a number of highlights, such as: the presentation of the working programme 2004 to 2007 of all 17 ISTA Technical Committees and Task Forces; a proposal for a Rules Chapter for GMO testing, for discussion and vote for inclusion to the ISTA Rules by the ISTA voting delegates; a presentation of the results of the worldwide ISTA GMO proficiency tests; a presentation and discussions on the accreditation process for laboratories wishing to issue ISTA Certificates for GMO testing, to be incorporated from 2006 on; a presentation and discussions on the new layout of the ISTA Rules, which should enhance the user friendliness of the ISTA Rules; and a discussion and decision on the increase of the seed lot size for cereal seed lots.



## GENERAL INFORMATION

### Getting There

Bangkok is a major travel hub, so it has plenty of direct flights from capital cities in Asia, Australia, Canada, Continental Europe, the UK and USA. The Airports Authority Of Thailand operate the Bangkok International Airport, located 25km north of the city center. It is a major gateway in South-East Asia and serves inbound and outbound flights daily.

### Getting Around

Getting around Bangkok may be difficult for the uninitiated, but once you're familiar with the transport system the whole city is accessible. The main obstacle is traffic, which moves at a snail's pace during the day. This means advance planning is a must when you're attending scheduled events or making appointments. If you can, avoid the traffic and travel by river, canal or Skytrain.

The BTS Skytrain is Bangkok's elevated rail system, providing clean, user-friendly rail

travel with great views in the bargain. Trains run frequently along two lines from 6am to midnight and are labelled with their final destination. Free maps of the system make life even easier.

Look for taxis with signs on top reading 'Taxi Meter' as these are always cheaper than non-metered taxis. It's a good idea to carry your hotel's business card with you as it will have directions written in Thai if you are having difficulty finding your way home. Make sure you agree on a fixed fare if you take a non-metered taxi or túk-túk.

River taxis are a cheap, convenient and work a regular route along the Mae Nam Chao Phraya. Bangkok Metropolitan Authority operates two canal routes: Khlong Phasi Charoen and Khlong Saen Saeb in Thonburi.

### Airport Transfer to Hotel

Participants are encourage to arrange their own transportation from the airport to the hotel - with either Airways Limousine, Airport Taxi Service or Public Taxi. Public Taxis are available at the airport at all times, the fare is charged according to the meter plus 50 Baht airport charge.

### Visa Application

The Secretariat will send out letters of invitation to participants upon written request. However, it should be understood that this letter is only to help delegates to raise travel funds or to obtain a visa, and is not a commitment on the part of the organisers to provide any financial support.

Delegates requiring invitations for visa applications must prepay registration before the invitation letter will be issued. Requests for visa invitation letters must be sent to the Secretariat by fax. Please take into consideration that the Secretariat will NOT deal directly with the Embassies for Visa requests for single participants.

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

For information or to  
register online visit

[www.seedtest.org](http://www.seedtest.org)



## Special Offer for New ISTA Members

Combine your registration for the ISTA Ordinary Meeting 2005 with your  
ISTA Membership application!

By combining your registration for the upcoming ISTA Ordinary Meeting 2005 with a  
*personal or laboratory* ISTA Membership, you can profit double:

- you can profit immediately from the reduced ISTA Member registration fee for the Ordinary Meeting 2005
- you get a **reduction of 20%** on the ISTA Membership Subscription fee for the year 2005\*

## Become an ISTA Member Now!

\*This offer is only available to new ISTA Members

### PRELIMINARY PROGRAMME

#### SUNDAY APRIL 24, 2005 REGISTRATION

16:00 - 18:00 Registration of Participants at Sofitel Central Plaza Hotel

#### MONDAY April 25, 2005 SESSIONS Presentation of Working Programmes, activities and special projects

08:00 - 18:00 Registration of Participants at Sofitel Central Plaza Hotel

08:45 - 09:00 Opening Ceremony

09:00 - 09:30 Bulking and Sampling Committee Session

09:30 - 10:00 Purity Committee Session

10:00 - 10:30 Germination Committee Session

10:30 - 10:45 Coffee break

10:45 - 11:15 Tetrazolium Committee Session

11:15 - 11:45 Vigour Committee Session

11:45 - 12:15 Moisture Committee Session

12:15 - 13:15 Lunch

13:15 - 13:45 Editorial Board Session (Seed Science & Technology)

13:45 - 14:15 Statistics Committee Session

14:15 - 14:45 Seed Health Committee Session

14:45 - 15:15 Proficiency Test Committee Session

15:15 - 15:30 Coffee Break

15:30 - 16:00 Variety Committee Session

16:00 - 16:30 Flower Seed Committee Session

16:30 - 17:00 Forest Tree and Shrub Seed Committee Session

17:00 - 17:30 Nomenclature Committee Session

17:30 - 18:00 Seed Storage Committee Session

#### TUESDAY APRIL 26, 2005 SESSIONS

##### Discussion on current important issues

09:00 - 10:30 GMO Testing issues:  
1. Proposal for a Rules Chapter for GMO testing

10:30 - 10:45 Coffee Break

10:45 - 11:15 2. Results of the ISTA GMO Proficiency Tests

11:15 - 12:15 3. Accreditation of GMO testing laboratories

12:15 - 13:15 Lunch

13:15 - 14:15 Amalgamation of Rules and Annexes

14:15 - 15:15 Accreditation Session

15:15 - 15:30 Coffee Break

15:30 - 17:00 Rules Committee Session

### WEDNESDAY APRIL 27, 2005 ORDINARY MEETING

09:30 - 10:30 Opening Ceremony

10:30 - 11:00 Coffee Break

11:00 - 12:30 Ordinary Meeting (Block 1)

1. Call to order
2. President's address
3. Roll call of Designated Members entitled to vote
4. Reading and acceptance of Minutes

Lunch

12:30 - 13:30 Ordinary Meeting (Block 2)

13:30 - 15:00

5. Report of the Executive Committee
6. Report of the Secretary General (incl. Presentation of Multi-Annual Budget)

15:00 - 15:30 Coffee break

15:30 - 16:30 Ordinary Meeting (Block 3)

7. Discussion on Governance of the Association - Membership; Membership subscription fee and voting rights
8. Constitution changes

16:30 - 17:15

19:30

#### Official Dinner

### THURSDAY APRIL 28, 2005 ORDINARY MEETING

09:00 - 10:30 Ordinary Meeting (Block 4)

9. Consideration and Adoption of the proposed Rules Changes 2005

Coffee Break

10:30 - 11:00 Ordinary Meeting (Block 5)

9. Consideration and Adoption of the proposed Rules Changes 2005 [cont.]

11:00 - 12:00 Lunch

12:00 - 13:00 Ordinary Meeting (Block 6)

10. Announcement of the place and date for the next Ordinary Meeting of the Association
11. Any other business raised by a Member, of which notice in writing has been received by the Secretary General two months prior to the date of the meeting
12. Any other business raised by consent of the Executive Committee

13:00 - 14:30 Coffee break

14:30 - 15:00 Ordinary Meeting (Block 7)

15:00 - 16:00

13. President's closing address
14. Adjournment

# ISTA GMO Proficiency Tests

## Use of presence/absence (qualitative) results for an overall rating from more than one test

By **Christoph Haldemann**, ISTA GMO Task Force Member, Swiss Federal Research Station for Animal Production, Switzerland, e-mail: c\_haldemann@yahoo.de and **Sylvain Grégoire**, ISTA Statistics Committee Chair GEVES, France, e-mail: sylvain.gregoire@geves.fr



Christoph Haldemann



Sylvain Grégoire

### 1. Introduction

The aim of this article is to show how **presence/absence** results of Proficiency Test (PT) with known spiking levels of GM seeds could be managed; using the system which is already in use in ISTA for other types of tests (Purity, Germination, etc.).

Up to now ISTA GMO TF carried out 3 Proficiency Tests; participating laboratories had to detect the presence of GM seeds in samples of conventional seed of corn, (*Zea mays* L.).

In this article both

- a conventional seed sample wrongly reported by the laboratory as containing GM seeds,
  - a sample containing GM seeds wrongly reported by the laboratory as containing no GM,
- are considered as a miss-classification (an error).

With this article we use the number of misclassified samples, and suggest 3 rating systems, one compares with an absolute number of miss-classifications, and 2 others compare with a relative number (a percentage) of miss-classifications.

Actual data from the laboratories which participated in all three ISTA Proficiency have been used. This is a total of 21 laboratories, as not all laboratories participated to the 3 tests.

### 2. Design of the three ISTA Proficiency Tests

The laboratories received samples with corn seeds (*Zea mays* L.) only.

Some samples contained only conventional seeds, for these samples the expected result was "absence of GM seeds".

Other samples of conventional seeds (non GM seeds) were spiked with GM seeds, for these samples the expected result was "presence of GM seeds".

### Proficiency Test 1

Each laboratory received 30 samples containing about 300 seeds.

Composition of samples:

- 12 negative samples
  - 6 positive samples, 1%; Mon810 only (3 seeds)
  - 6 positive samples, 1%; T25 only (3 seeds)
  - 6 positive samples, 1%; Mon810 + T25 (1 seed of Mon810 + 2 seeds of T25)
- 43 laboratories participated.

### Proficiency Test 2

Each laboratory received 10 samples containing about 3000 seeds.

Composition of samples:

- 3 negative samples
  - 3 positive samples, 0.7%; Mon810 only (21 seeds)
  - 4 positive samples, 1.4%; Mon810 only (42 seeds)
- 50 laboratories participated.

### Proficiency Test 3

Each laboratory received 12 maize samples containing about 1500 seeds.

Composition of samples:

- 3 negative samples
  - 3 positive samples, 0.2%; Mon810 + T25 (1 seed of Mon810 + 2 seeds of T25)
  - 3 positive samples, 2%; Mon810 + T25 (10 seed of Mon810 + 20 seeds of T25)
  - 3 positive samples, 4%; Mon810 + T25 (20 seed of Mon810 + 40 seeds of T25)
- 40 laboratories participated.

### 3. The current ISTA system to rate a Proficiency Test, and a run of 6 tests

ISTA Proficiency Tests overall rating procedure is described below (Table 1).

One test rating	One test Score Value	Overall rating on 6 tests	Range on 6 tests <sup>5)</sup>
<sup>1)</sup> A	5 points	<sup>1)</sup> A	28 - 30 points
<sup>2)</sup> B	4 points	<sup>2)</sup> B	21 - 27 points
<sup>3)</sup> C	3 points	<sup>3)</sup> C	16 - 20 points
<sup>4)</sup> BMP	0 points	<sup>4)</sup> BMP	below 16 points

Table 1: ISTA rating system for 6 Proficiency Tests based on the in-round rating values.

Depending on the results obtained by a laboratory on a given proficiency test, the laboratory is rated A, B, C or BMP. There is no official definition of A, B, C and BMP in the case of GM tests, the definitions below are an attempt to show the philosophy of the system which can be applied to many types of tests:

To each rate correspond a number of points, the bigger number the better the results. The decrease from A to C and the "0" for BMP, are another way to understand the meaning of these rates.

The rating for a given Proficiency Test is an indication to the lab on its performance in this test.

For laboratories which have an ISTA Accreditation on a type of test, participation to Proficiency Tests is compulsory. The set of the 6 most recent tests is used in the evaluation process. The sum of points collected on the tests, is compared on the range in last column of Table 1.

<sup>5)</sup> Range for 6 Proficiency Tests

For instance a lab with a total of 23 points on 6 tests will be rated B on the set of 6 tests.

In this article we use exactly this system, but as we have only 3 tests available we use a "Range 2", which is the usual range divided by 2.

This range 2 shall not be used in the current ISTA system, but will allow us to describe the mechanism of the proposal (as soon as 6 tests are available, the current ISTA system can be applied).

rating	score	Overall rating	Range <sup>5)</sup>	Range 2 <sup>6)</sup>
<sup>1)</sup> A	5	<sup>1)</sup> A	28 - 30	<b>14 - 15</b>
<sup>2)</sup> B	4	<sup>2)</sup> B	21 - 27	<b>11 - 13</b>
<sup>3)</sup> C	3	<sup>3)</sup> C	16 - 20	<b>8 - 10</b>
<sup>4)</sup> BMP	0	<sup>4)</sup> BMP	below 16	<b>below 8</b>

<sup>1)</sup> A No problem has been detected in this test.

<sup>2)</sup> B There are small problems, but no specific look or action is suggested to the participant.

<sup>3)</sup> C Problems, ISTA indicates there might be things to consider by the laboratory to explain or correct things.

<sup>4)</sup> BMP Below Minimum of Performance, ISTA indicates by a letter that the results were poor and the laboratory has to explain and correct things.

<sup>6)</sup> Range 2 is for Proficiency Tests in this article

**Table 2:** Range 2, used to show the mechanism with only three tests available.

In the current ISTA system, the mechanism to derive a A,B,C or BMP from the raw results of the test may differ depending on the type of test. The computations are for instance not the same to check Germination, and for the retrieval of spiked seeds of other species.

For presence/absence of GM seed, there is not yet an official computation system in place in ISTA. The following proposals show possibilities that have been considered by the Proficiency Test sub-group of the ISTA GMO TF in conjunction with the ISTA Statistics Committee.

**4. Proposal of a rating system for presence/absence of GM seeds in conventional seeds**

The Proficiency Tests carried out by ISTA on detection of GM seeds in conventional seeds are prepared with great efforts to ensure that the expected result (presence or absence) of each sample is very sure.

This can be compared to the spiking of seeds from other species in seeds from a species, as carried out for other seeds determination. This is different from the situation of germination, where the number of normal seeds in a given sample can not be known in advance.

The proposals are based on counting the "mistakes". Two types of mistakes can occur:

- a conventional seed sample is wrongly reported by the laboratory as containing GM seeds,
- a sample containing GM seeds is wrongly reported by the laboratory as containing no GM seeds.

The total number of miss-classified samples is used as a basis for the rating of a given Proficiency Test.

**Rating calculated with an absolute number of misclassifications (Rating System 1).**

In this system the number of misclassifications is used, and a table gives the correspondence to the rate as shown below in Table 3.

rate	Number of misclassified samples
A	0 errors
B	1 or 2 errors
C	3 errors
BMP	more than 3 errors

**Table 3:** Rating system 1

**Rating calculated with relative (percentage) errors (Rating System 2 and Rating System 3).**

The principle for the calculation remains the same, counting the number of samples misclassified. But instead of working with an absolute number of errors, the errors are computed in percent of all samples received for each given Proficiency Test, and compare to the right column (Percentage of misclassified samples) shown in Table 4 and Table 5.

Two proposals are described in Table 4 and Table 5.

rate	Number of misclassified samples
A	0% - 5%
B	>5% - 10%
C	>10% - 20%
BMP	>20%

**Table 4:** Rating system 2

rate	Number of misclassified samples
A	0% - 6%
B	>6% - 20%
C	>20% - 30%
BMP	>30%

**Table 5:** Rating system 3

*ISTA GMO Proficiency Tests is continued on page 10*

### 5. Results

The number of misclassifications (Y axis) for each laboratory is summarised in Graph 1 for the 21 laboratories (X axis) which carried out the 3 Proficiency Tests.

See Graph 1

13 laboratories made no misclassification. 8 laboratories had some misclassifications in one, two, or the three Proficiency Tests. PT1, PT2, PT3 refers respectively to the first, second and third ISTA Proficiency Test.

The 3 proposed rating systems have been applied to each of the 3 Proficiency Tests separately. Then the sum of points has been compared to Range 2 in Table 2, to obtain an overall rate on 3 tests. The 4 overall rates (A,B,C and BMP on X axis) is shown in graph 2, where the Y axis is the number of labs (out of 21) in each rate category for each rating system (black for rating system 1, red for rating system 2, green for rating system 3).

See Graph 2

16 to 18 labs obtained an overall A. With the range applied, and the current ISTA system, an overall A can be obtained even if not all individual tests are A.

In this article a 14 can be obtained with 2 A and 1 B resulting in an overall A. (A is obtained if the sum of points is 14 or 15)

### 6. Discussion

The three proposed rating systems give rather similar outcomes.

The figure in Graph 2 is rather similar to graphs shown at Budapest Congress on other types of tests.

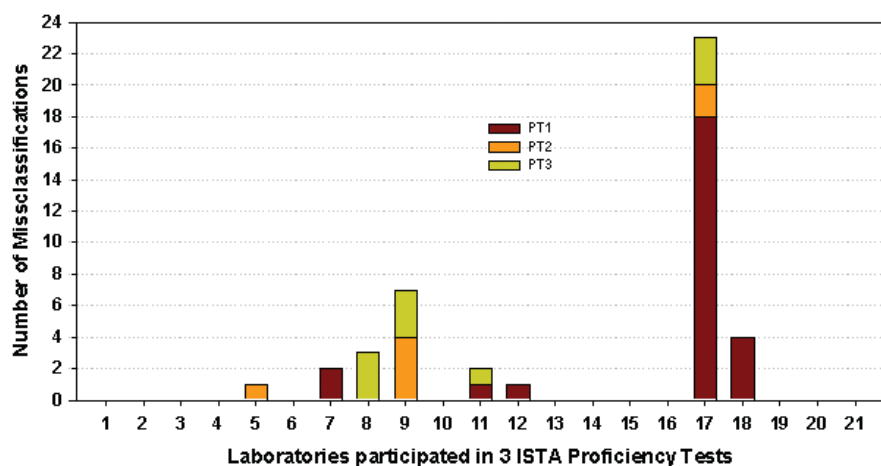
If we look at the most crucial score class BMP (Below Minimum Performance), one or two labs are pointed out, depending on the rating system. It make sense that a lab with mistakes in all the three tests and 23 misclassifications in total is ranked as BMP.

Only a few laboratories had problems to detect the presence or absence of GM seeds in samples of conventional seed of corn (*Zea mays* L.). Some participating laboratories are already very well experienced, while some other laboratories may have participated to the tests with yet few or not enough experience, as a way for them to check their progress.

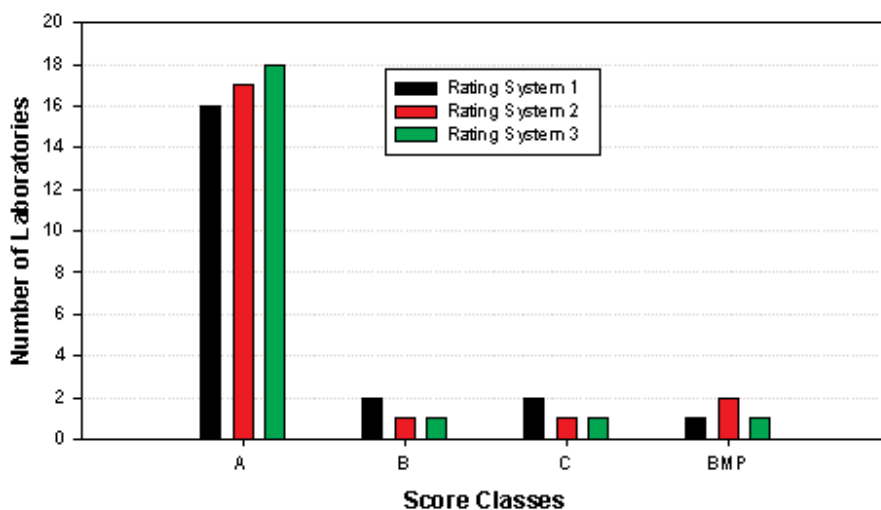
The values used to derive a rate from misclassifications (Number of misclassified samples in Table 3, respectively Percentage of misclassified samples in Table 4 and 5) are possibilities among hundreds. The same idea can be kept, and the numbers changed if necessary in the future. These values have been selected not only using the 3 ISTA Proficiency Tests, but also from discussions with laboratory experts.

These proposals are under evaluation by ISTA.

The same question of rating procedure is also under development for the rating of results expressed as a quantity of GM material (% seeds, % DNA copies, etc.) The general idea has been shown at Budapest Congress; a short description of the proposals will be published in a next issue. ■



Graph 1: Total errors for PT1, PT2 and PT3 (30 + 10 + 12 = 52 samples)



Graph 2: Comparison of the 3 Rating Systems for the laboratories participated in all 3 ISTA Proficiency Tests (21 laboratories)

## Announcement 4<sup>th</sup> ISTA Proficiency Test on GMO Testing

After performing three rounds on *Zea mays* L. there will be a change in the species for the 4<sup>th</sup> round to Soybean (*Glycine max* (L.) Merr).

Laboratories interested in participating should please contact the ISTA Secretariat:

E-mail: [ista.office@ista.ch](mailto:ista.office@ista.ch)

Fax: +41 1 838 60 01

More details can be found in the Announcement posted on the ISTA Website at [www.seedtest.org](http://www.seedtest.org)

Please find more details about the Proficiency Test on page 48.

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MicroScience

# Organic seed treatment to control common bunt (*Tilletia tritici*) in wheat

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## Abstract

Common bunt caused by the fungus *Tilletia tritici* (syn. *T. caries*) is one of the most devastating plant diseases in wheat. In conventional agriculture the disease is controlled exclusively by fungicide seed treatment, but in organic farming these fungicides are not accepted. Previous studies in India have shown that seed treatment with plant extracts of *Canabis sativa*, *Eucalyptus globulus*, *Thuja sinensis* and *Datura stramonium* was fully effective against the disease under field conditions. Later, in vitro studies have shown that also germination of spores of the Karnal bunt pathogen (*Neovossia indica*) could be prevented by these plant extracts. The experiment was repeated in Denmark with extracts from the same species grown in Denmark, which has climate conditions very different from India. In this experiment, the same seed treatments had no or very limited effect on the frequency of the disease. The treatments were compared with indigenous methods from Europe including salty brine, *Thuja* leaves and lime. These methods had a significant, but insufficient effect on disease suppression.

## Introduction

Common bunt (*Tilletia tritici* syn. *T. caries*) is also called stinking smut and in India it is called hill bunt. In conventional agriculture in Europe, common bunt is one of the diseases most intensively treated with pesticides, and about 80-90% of all seed lots of winter wheat in industrialized agriculture are treated with synthetic fungicides (Nielsen *et al.* 1998). In the arid zones of less industrialized agriculture, common bunt is still one of the diseases causing most devastating yield losses of up to 30% in some areas (Mamluk 1998). In organic agriculture common bunt is a difficult disease to control in the absence of fungicides (Borgen 2000).

Singh *et al.* (1979) were able to control the infection of common bunt by 100% by soaking wheat seed in plant juices of *Canabis sativa*, *Eucalyptus globulus*, *Thuja sinensis* and *Datura stramonium*. In order to develop a strategy to control the disease in organic agriculture, the most promising treatments found by Singh *et al.* (1979) were included in

a series of treatments in control of common bunt under Danish cropping conditions.

Two thousand years ago, Pliny the Elder (Caius Plinius Secundus) wrote in his *Historia naturalis* that by mixing cypress leaf into the seed lots, a significant plant disease could be controlled. It is likely that this plant disease was common bunt (Buttress and Dennis 1959). The recommendation was repeated in the Almanac during 16th and 17th century in Denmark (Olsen 1791), but at this time conifers were rare trees in Denmark, and in stead Olsen (1791) recommended seed treatment with lime to control common bunt.

During the 16th century a seed treatment against common bunt was developed by soaking seed into salty water (Woolmann and Humphrey 1924, Buttress and Dennis 1959). Soaking seeds into water or plant juices will increase the water content of the seeds to an extent, where re-drying is required for storage and for sowing with a conventional sowing machine. The drying process is expensive and energy consuming especially in temperate climatic zones, and the proposed designs, where seeds are soaked into a liquid, are therefore not optimal for modern organic agricultural practice in Denmark. The aim of the present study is to investigate the potential of different classic seed treatments to control common bunt in a design applicable to the practice in organic wheat production in Denmark.

## MATERIALS AND METHODS

The seeds of the variety Kosack were contaminated with 5 g spores of *Tilletia tritici* per kg seeds, which resulted in a contamination of  $1.7 \cdot 10^6$  spores per gram seeds when tested by the ISTA haemocytometer method (Kietreiber, 1984).

Singh *et al.* (1979) treated seed by soaking the seed for 15 minutes into juices of different plants. By this treatment spores of the pathogen will be washed off the seeds. It is therefore not possible to conclude to which extent the effect of the treatment is a washing effect or a chemical effect. To investigate this, a different design was chosen. The species *Thuja sinensis* described by Singh *et al.* (1979) is unknown to the taxonomy used in

Denmark, but the common name Chinese Thuja is used for *Thuja occidentalis* in some countries and this species was chosen for the experiment in the hope that they are closely related or even synonyms for the same species. *Thuja occidentalis*, *Canabis sativa* and *Datura stramonium* were grown in open air in Denmark, while *Eucalyptus globulus* were grown in a green house. Leafs of the plants were put into a cylinder ( $\phi=10$  cm,  $h=10$  cm) with 1 mm holes and pressed under 20 tons. The collected juices were filtered in a sieve with 0.2 mm holes. Seeds were treated in a spinning wheel seed dresser (Hege no. 11) in a dose of 30 ml/kg of the concentrated juices.

Leaf/needles of *Thuja occidentalis* and *Picea glauca* were dried in an oven for 2 hour at 80 °C and grinded into meal. Seeds were then by turn added water and meal until 42 g meal adhered per kg. However, a part of the meal may have fallen off later during seed handling and sowing, since no other adhesive were used. To further investigate the mode of action, treatments were included, where pure oils of *Eucalyptus* and *Pinus* were added in the seed dresser in a dose of 18 ml/kg.

Olsen (1791) recommended a lime treatment where a pile of seed was sprinkled with slaked lime. In the present study a design was chosen, where powder of quick lime (Calcium hydroxide) were mixed with water (2:3) (quick lime turns into slaked lime when mixed with water). 100 ml of this liquid was added per kg, and on top of it 30 g per kg of quick lime powder.

Each seed treatment was repeated 5 times (true replicates), one for each field replicate and one for germination test. After treatment the seeds were stored at 5 °C. Samples were removed for sowing of field tests 4 days after seed treatment. Germination tests were conducted 1 month later.

Field trials were conducted at Højbakkegård, an experimental farm of the Royal Veterinary and Agricultural University, on Zealand, Denmark. In the field trial seeds of each treatment were sown in 4 replicates in 6 m<sup>2</sup> plots of a rate of 400 seeds per m<sup>2</sup>. After heading the number of infected ears were counted based on visible macro-symptoms. In average, 2000 plants in each plot were assessed for common bunt infection in each treatment.

Germination tests were conducted as cold sand-tests, testing the germination speed of the treatments (Borgen 2000, Borgen and Kristensen 2001, Borgen and Nielsen 2001). Results were analysed by a Generalised Mixed Model (GENMOD, software SAS ver. 8.01).

## Results and discussion

The effect of the different treatments is listed in Table 1.

**Table 1.** Effect of seed treatments to control of common bunt (*Tilletia tritici*).

Treatment	Dose ml/kg	Diseased heads	Days for 50% germination
Control		53.3%	9.6
Water	30	46.4%	9.5 n.s.
Salty water	30	30.9% n.s.	10.5 (p=0.0051)
Quick lime (see text)		10.9%***	9.0 n.s.
<i>Canabis sativa</i> , juice	30	52.2% n.s.	9.4 n.s.
<i>Eucalyptus globulus</i> , juice	30	52.3% n.s.	9.5 n.s.
<i>Thuja sinensis</i> , juice	30	53.0% n.s.	9.2 n.s.
<i>Datura stramonium</i> , juice	30	57.3% n.s.	9.0 n.s.
<i>Eucalyptus</i> , oil	18	48.9% n.s.	10.1 n.s.
<i>Pinus</i> , oil	18	46.0%*	11.1***
<i>Thuja sinensis</i> , meal	42g/kg	28.7%***	9.7 n.s.
<i>Picea glauca</i> , meal	42g/kg	30.2%***	9.5 n.s.

None of the plant juices used by Singh *et al.* (1979) could significantly reduce the frequency of common bunt in this study, even though they had been 100% effective in their trials. Gupta and Singh (1983) found *in vitro* that plants extracts of the same species inhibited spore germination of the related species *Neovossia (Tilletia) indica*, when spores were soaked in the extracts for 5 days, except for the *Eucalyptus*-treatment, which did not reduce, but instead enhanced the germination

of the spores. Sharma and Basandrai 1998 found some reducing effect *in vitro* of *Canabis sativa* and *Eucalyptus tereticornis* when germinating spores of *N. indica* in extracts from boiled leaves of the plants.

There may be different explanations for the contra-dictionary effects of the plant extracts on the development of the bunt disease. The plants used in the trials were different varieties grown under extremely different conditions. The content of primary and secondary metabolites is therefore likely to be very different, and most likely the content of most metabolites are higher in wild plants grown in the Simla hills in India than cultivated and fertilized varieties grown in Denmark, especially in a green house. The design in the treatment procedure was very different, since the seed were not soaked into the liquid in the experiment presented here. The effect of the soaking treatment presented by Singh *et al.* (1979) can not be a washing effect alone, since other plant juices and deluded plant juices had less or no effect on the bunt frequency. However, the soaking treatment may have other effects on the plant-pathogen interaction, which may explain the difference in effect.

*Cyprinus sempervirens* was the only *cyprinus* species grown in the Roman Empire, and it is therefore likely that this is the species recommended by Pliny to be mixed into the seed lots. *Thuja sinensis* and *Cyprinus sempervirens* are closely related species, and applying leaf meal to the seeds significantly reduced bunt frequency, as was the case with meal of *Picea glauca*. It is therefore likely that the recommendations by Pliny and in the Danish Almanacs from the 16th and 17th century actually had an effect on the bunt frequency, even though the effect is not complete in the current study.

Lime used as a seed treatment reduced the bunt frequency by 80%, and the treatment was frequently used in the 17th and 18th century in Europe (Woolmann and Humphrey 1924, Buttress and Dennis 1959). The results indicate that lime can be used to reduce bunt frequency, but in the current design the treatment did not offer a complete control.

Common bunt is a very serious disease, reducing not only yield, but also grain quality. Only a single infected plant per m<sup>2</sup> will make the whole harvested crop smell like rotten fish, and unacceptable to commercial wheat production. It is therefore crucial for the seed propagation that common bunt is under complete control. Some of the classic methods examined in this study reduced the bunt frequency significantly, but could not control the disease sufficiently. These treatments can therefore not be used alone in cases of very

high spore load of the seeds.

To control common bunt in organic agriculture it is recommended to use a combination of different measures. This includes discarding the most infested seed lots, use of resistant varieties and removal of spores from the seed e.g. by brushing. On top of this strategy, seed treatments can be used (Borgen 2000). As seed treatment for organic agriculture it is recommended to use mustard or milk powder (Borgen and Kristensen 2001), milk-powder in combination with bio-agents (Borgen and Davanlou 2000), acetic acid (Borgen 2001) or hot water treatment (Nielsen *et al.* 2000), which are more efficient than the ones tested in this experiment. With a combination of these tools, common bunt can be controlled in organic agriculture in the future. ■

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# Healthy seeds, the basis for sustainable farming



Paper Series from the 4<sup>th</sup> ISTA PDC Symposium on Seed Health

Wageningen, Netherlands, April 29 - May 1, 2002

The ISTA Seed Health Committee continue with the new series in the Seed Testing International. The two papers 'Use of water restriction technique in seed pathology' by J. C. Machado; R. M. Guimaraes; M. G. G. C. Vieira, R. M. Souza, Brazil, and 'Varietal difference between artificial and natural infection of Ustilago avenae. The importance of size of kernels and main location of infection' by Karin Sperlingsson, Sweden are the third in a series of papers related to the field of seed health and show the technical and scientific work of our Association.

The papers are works which were presented at the 4<sup>th</sup> ISTA PDC Seed Health Symposium 'Healthy seeds, the basis for sustainable farming' held in Wageningen, The Netherlands, April 29<sup>th</sup> to May 1<sup>st</sup>, 2002.

The ISTA PDC Symposia (now the Seed Health Committee Symposia after the renaming of the PDC) are used as a platform to exchange ideas, new techniques, and information in the various topics of seed health. The high profile of seed health will be reflected in the future presentations in this series covering regional seed health issues, quality management, and innovations and new methods in seed health testing.

See page 37 for the Announcement of the 5th ISTA Seed Health Committee Symposium

## Use of Water Restriction Technique in Seed Pathology

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### Introduction

The central core of this paper is to report on the applicability of water restriction technology in seed pathology and to present some results of research developed at the Federal University of Lavras (UFLA)-Br on this application. The application of the water restriction technique is considered in five distinct areas of interest in seed pathology: i) to obtain infected seeds, ii) to control seed germination in seed health testing, iii) for studies on relationships between inoculum potential and seed performance, iv) to simulate drought conditions, and v) to separate healthy seeds from infected seeds in naturally contaminated lots. Results of some research developed at UFLA on this technology are briefly presented and described with the focus on the major pathogen-seed pathosystems.

### Concept and basis of the water restriction technique in relation to seed pathology

The control of the germination process is essential in many circumstances involving seed management and this control can be performed in different ways. In some cases, complete inhibition of germination is the goal and in others the aim is to reduce the radicle elongation to a certain extent.

Under the scope of the Seed Technology, the water restriction technique is intended to control the germination process and to improve the final quality of a seed lot. This is accomplished by means of osmotic modifications of substrates on which seeds are placed for germination. Under these circumstances, seeds are conditioned to initiate their germination process, to undergo phases I and II, without any radicle protrusion for several days of treatment. This process is known as 'osmo-priming' or 'seed pre-conditioning' (Heydecker, Higgins & Turner, 1975).

After the pre-conditioning treatment, seeds

are able to germinate normally when favourable conditions are provided. In addition, pre-conditioned seeds of many species can be dried and stored for some time (Del Giudice *et al.*, 1999).

Osmotic potentials of filter paper or gel substrates can be controlled using polyethylene glycol (PEG) or other osmotic compounds like salts or other solutes (Prisco and O'Leary, 1970; Eira, 1988; Guimarães, 1991; Braccini, 1996; Camargo, 1998). In Table 1 quantities of some common osmotic compounds are indicated to prepare solutions and agar substrates. The quantities of each solute are calculated using software SPPM (Michel & Radcliffe, 1995).

**Table 1. Quantities of chemicals used in the preparation of solutions and agar**

A - Osmotic Solution			
Osmotic potentials (MPa)	Osmotic compounds (g L distilled water)		
	NaCl	KCl	Mannitol
-0.6	7.71	9.94	44.88
-0.7	9.02	11.65	52.33
-0.8	10.33	13.36	59.75
-0.9	11.64	15.08	67.23

B - Osmotic PDA medium			
Osmotic potentials (MPa)	Osmotic compounds (g L distilled water)		
	NaCl	KCl	Mannitol
-0.6	3.10	4.00	18.42
-0.7	4.40	5.61	25.73
-0.8	5.71	7.32	33.10
-0.9	6.91	8.93	40.44

medium with different osmotic potentials.

From the Seed Pathology point of view, complete or partial inhibition of seed germination is of great interest in the fields of Seed Health Testing and Seed Infection Understanding. The development of methods



that impedes or reduces seed germination without affecting microorganisms associated with seeds are therefore the concern of the seed pathologists. In that direction, water restriction technology, as it has been developed and used by seed technologists to improve seed quality, is a very promising technique. In earlier investigations, water restriction did not affect fungal development to a great extent. The procedure may be easily managed to control the germination process for seeds of most plants and for growth of fungal species.

From available reports fungal development may vary largely in relation to osmotic potentials of substrates; but they can be affected only at higher potential levels (Duniway,1979; Gao & Shain, 1995; Alam, Joyce & Wearing, 1996). In general, development of fungi are not reduced by osmotic potentials lower than - 2.0 MPa. At potentials in the range of -0.3 to -1.0 MPa growth of some fungi are stimulated (Adebayo and Harris,1971; Wearing and Burgess,1979; Subbarao, Michailides and Morgan,1993; Gao and Shain,1995; Carvalho, 1999 Costa, 2000; Machado, 2002.)

**Potential Applicability of Water Restriction in Seed Pathology**

**I - Obtaining infected seeds**

The availability of infected seeds in seed pathology is important for many purposes. Infected seeds are necessary in the evaluation of seed treatments, in the development and conduction of seed health testing, in epidemiological studies, and for teaching (Carvalho,1999; Costa, 2000). Infected seeds can be obtained from naturally infected lots and by infecting seeds through artificial methods. In both cases, difficulties exist in terms of operational aspects and seasonal variation. In most available methods, contamination is the mechanism mostly involved and this provides a low percentage of infection. Inoculation by the contact of seeds with fungal colonies in an agar substrate (PDA or ME) as investigated by Tanaka *et al.* (1989) has certain limitations. The exposure of seeds to a wet substrate during the time required for infection, i.e. longer than 36 h, may cause damage to seeds and these seeds can not be stored. Inoculation of mother plants in the field must be conducted during particular environmental conditions. This technique is subject to escapes and may result in the production of abnormal or few infected seeds.

the last 8 years, the water restriction technique has proved extremely useful to obtain infected seeds in a large number of cases. By this technique, seeds are maintained in direct contact with developing colonies of the pathogenic fungi or bacteria on agar media. Usually PDA for fungi and selective nutrient agar for bacteria are used and amended with an osmotic compound, such as mannitol, at potentials that vary according to the seed-pathogen combinations. (Carvalho, 1999; Costa,2000; Kobayasti, 2002; Machado, 2002); Examples of successful application of the osmo-technique to infect seeds are given in Table 2 and Figure 1. To obtain infected seeds with different potentials of a certain fungus, seeds should be exposed to the developing colonies for different periods of incubation. Successful results have been observed for *Colletotrichum gossypii* var. *cephalosporioides* in cotton (Machado *et al.*, 2002). In the case of bacteria, from the results of research conducted at UFLA on infecting bean seeds by *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* there is a positive expectation about the advantages of

using water restriction technique (Kobayasti, 2002).

The variation in percentages of infection for each pathogen-seed combination is mostly due to the physiological conditions of the seed in the lot being used, the period of exposure of seeds to pathogens, and the levels of tolerance to pathogens exhibited by the varieties of seeds inoculated. The natural occurrence of other microorganisms in the seed lot may be another factor that reduces the percentage of infection by the pathogens inoculated. Then, the pre-selection of a heal-



**Fig. 1. Anthracnose symptoms in common bean plants produced by inoculation of seeds with *Colletotrichum lindemuthianum* through water restriction technique**

**Table 2. Mean ranges of percentages of infected seeds of cotton, bean, maize and soybean after inoculation with some pathogenic seed-borne fungi through the osmo-priming technique in comparison with conventional technique.**

Host/Fungi	Infected seeds (%)	
	Conventional technique*	Osmo-priming technique**
<b>Common bean</b>		
<i>Colletotrichum lindemuthianum</i>	40-50	50-70
<i>Fusarium oxysporum f.sp. phaseoli</i>	30-40	40-60
<i>Sclerotinia sclerotiorum</i>	30-50	40-70
<b>Cotton</b>		
<i>Botryodiplodia theobromae</i>	50-80	60-100
<i>Colletotrichum gossypii</i> (including <i>C. gossypii</i> v. <i>cephalosporioides</i> )	50-70	70-100
<i>Fusarium oxysporum f.sp. vasinfectum</i>	30-40	50-70
<b>Maize</b>		
<i>Acremonium strictum</i>	10-20	20-40
<i>Diplodia maydis</i>	30-40	50-70
<b>Soybean</b>		
<i>Colletotrichum truncatum</i>	50-80	100
<i>Phomopsis sojae</i>	50-70	80-100

From results of research conducted at the Federal University of Lavras (UFLA) over

\*) Seeds on PDA and incubation of 36 h;  
\*\*) Seeds on PDA + Manitol or NaCl with incubation of 96-144 h.

thy seed lot is an important requirement for a successful inoculation. Reproduction of typical symptoms in some cases, like wilting caused by pathogenic species of *Fusarium* in bean and cotton, has not been achieved so far. This demands additional investigation on the infection mechanisms involved in these interactions.

It is also wise to consider that the osmo-priming technique provides conditions for seeds to germinate faster even when they are submitted to inoculation by exposure to fungal colonies in an agar medium modified osmotically. In this case, from statistical principles, non-inoculated seeds can be mixed with inoculated seeds to produce a desired seed lot. To be mixed, the non-inoculated seeds have to be also submitted to osmo-priming treatments in agar medium without fungal colonies. As a final remark, the improvement of the water restriction technique to infect seeds with some pathogens still demands a better understanding of the mechanisms involved in the infection process.

II - Seed Health Testing

In the seed health tests that require an incubation period, the germination of seeds can be a serious drawback for several reasons. Fast growing radicles produced during the incubation period may cause secondary contamination between seeds. If this contamination occurs, then the examination of germinating seeds for fungal structures may take longer than examination of non-germinated seeds (Machado and Langerak, 1993).

The inhibition of seed germination of dicotyledons species is made generally by the use of sodium 2,4-dichlorophenoxyacetate (2, 4-D) incorporated to the substrate in both blot-

ter and agar tests ( Hargbord *et al.*; 1950; Neergaard,1979; Machado,1988). For monocotyledons species and some small seeded dicotyledons killing of the seeds can be made by using deep-freezing following pre-imbibition in water (Limonard,1968; ISTA 1976; Neergaard,1979).

The use of 2,4-D and deep-freezing techniques in seed analysis laboratories may present some disadvantages, however. In addition to its toxicological property, 2,4-D may persist in laboratories as a chemical contaminant. Thus, this becomes a risk for other types of seed analysis such as for germination and vigour. Contrarily, the use of a deep-freezer may become impracticable if a high number of seed samples are to be run in a limited period of time and when fast growing saprophytes are also present in seeds. Commonly, the fast growing saprophytes on the dead seeds overgrow the pathogenic species during incubation period (Limonard, 1968). According to available reports, attempts to develop alternative techniques to replace 2,4-D and the deep-freezer methods in routine seed health testing have not been successful. However, the development of the water restriction method at the Federal University of Lavras is a promising technology to replace the 2,4-D and freezer methods in seed health testing for most cases. For most fungi, the incorporation of osmotic compounds, such as mannitol and NaCl at potentials of -0.6 to -1.0 MPa in blotter and agar substrates, makes examination of seed easier and more reliable over the conventional methods. Plant species for which use of the water restriction method has been successful are cotton, common bean, maize, soybean, wheat, and some vegetables. Illustration of the effect of mannitol on common bean and rice seeds is given in Figure 2.



Fig. 2. Effect of water restriction (mannitol) on the germination of common bean (top) and rice (bottom). Germinated seeds at left side refer to stand blotter test; seeds in the middle refer to modified blotter 2,4-D method for bean and the freezing method on rice. Seeds at right side in both cases refer to the osmotic treatment (Coutinho, 2000).

An interesting application of the osmotic principle in seed health testing was developed by Brodal (1997) to detect *Drechslera* species in cereal seeds. The incorporation of sucrose in solution used to moisten blotters provided conditions for the *Drechslera* species to change the colour of the substrate to pink-violet at the contact with incubated seeds.

By use of the water restriction technique, it was possible to develop non-destructive methods to detect some fungi and bacteria in seeds. The osmotic potential of the substrate

Table 3. Effect of natural inoculum density of *Fusarium moniliforme* on the performance of maize seeds. Essay carried out using water retriCTION technique. Lavras, MG, 2002

Inoculum Density	Germ	Stand		ESI	Plant size (cm)	Plant Weight (g)			
		Initial	Final			Up-ground portion		Under-ground portion	
						FW	DW	FW	DW
Zero	24.5 a	24.5 a	24.5 a	3.4 a	9.2 a	45.2 a	3.0 a	25.4 a	4.3 a
1 to 25%	23.2 ab	22.5 ab	23.2 ab	2.9 a	6.3 b	17.7 bc	1.3 b	8.9 b	1.3 b
26 to 50%	20.0 bc	20.2 bc	20.0 bc	2.9 a	7.1 ab	20.2 c	1.4 b	8.9 b	1.3 b
51 to 75%	18.0 c	18.0 c	18.0 c	2.6 a	5.8 b	12.6 bc	0.9 bc	6.9 b	0.9 b
76 to 100%	10.0 d	9.2 d	10.2 d	1.4 b	5.9 b	8.0 c	0.6 c	3.9 b	0.5 b
LSD	3.31	3.72	3.31	0.79	2.13	10.52	0.58	5.48	0.83
CV (%)	7.90	9.01	7.90	13.74	14.52	23.30	18.84	23.18	22.91

Means in the same column with similar letters are not significantly different at P=0.05, (Tuckey test) ESI= Emergence speed index, FW= fresh weight, DW= dry weight

should be such that the protrusion of the radicle is prevented but the development of pathogens on the seeds is not affected. This versatile water-restriction method can be used to test valuable seed lots, e.g., seeds in a germ plasm bank and transgenic material. After the water restriction test, the seeds can be dried and stored with little effect on their viability.

**III - Pathogen effects on seed performance**

The effects of pathogens on seed quality are variable and can be expressed during the germination period or later when seeds are planted in the field. According to the pathogen or group of pathogens, the damage depends on the potential and position of the inoculum in seeds and other factors. From a low level of infection, seeds can be protected or the pathogen may be arrested due to resistance mechanisms or escape. For high levels of inoculum, infection of seeds is likely to take place and this leads to disease transmission and dissemination of the pathogens in fields and between fields. To follow the actual effect of infective inoculum on seed performance, researchers make use of artificial methods of inoculation in which seeds are soaked in an inoculum suspension or mixed with inoculum as a dry formulation. In other cases, lots of contaminated seed with different levels of infection or contamination are submitted to controlled tests that provide an indication of the effect of the pathogens present in the seed lots. Through this technique, individual seeds can be followed for later examination of the pathogen effects.

An interesting point is that by means of the water restriction technique, seeds of the same lot can be separated into different classes of inoculum density after a period of time, and then followed after sowing in a separate way.

In this technique, a number of seeds are incubated on blotters moistened with an osmotic solution, normally mannitol at -1.0 to -1.2 MPa, for 6-7 days. Then, mycelial growth and intensity of sporulation are used to rank the inoculum potential that developed on seed and on ungerminated seeds. Successful application of this procedure has been used for *Bipolaris sorokiniana* in wheat (Fig. 3), *Fusarium moniliforme* in maize seeds (Table 3) and *Drechslera oryzae* in rice (Machado and Machado, 2002; Souza *et al*, 2000; Celano *et al*, 2002).

In all the cases, seed performance, is determined through evaluation of germination, vigour, plant stand, height and weight of emerged plants, and the disease index. All of the foregoing are affected proportionally to the density of inoculum in seeds.

From the results of studies conducted to date, variation exists between pathogens as far as inoculum density and potential is concerned. For example, in the case of *F. moniliforme* on maize seeds, the percentage of germination is only reduced statistically when the inoculum potential in seeds is very high; that is, the inoculum covers more than 50% of the seed surface. However, the weight of roots is severely reduced in seeds with even a low level of inoculum that covers the seeds. For pathogens, such as *D. oryzae* and *B. sorokiniana* (Fig. 3), the effects of an increased inoculum potential are more damaging to seed, even from low levels of initial inoculum.

**IV - Simulation of dry conditions**

The limitation of water supply in the substrate, blotters or other media, by use of the osmotic technique, dry conditions can be reproduced with success for several studies

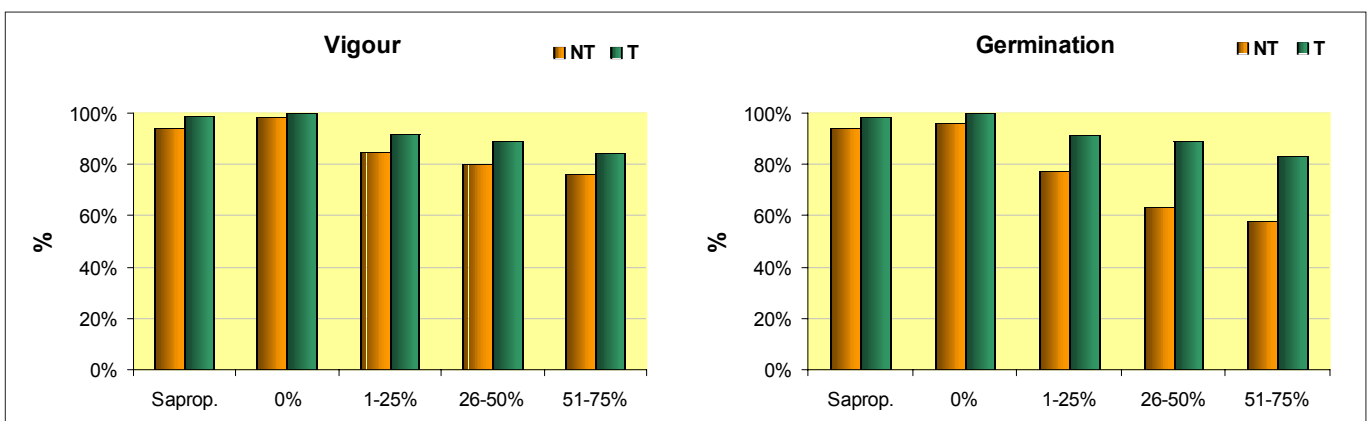
in the laboratory. This is important, for example, in research to evaluate seed treatment performance under drought conditions as it may occur in the open field. In practice, sowing seeds in wet soil followed by a prolonged dry period makes the seeds to be subject to numerous pathogens that are soil inhabitants, such as *Fusarium* sp., *Rhizoctonia solani*, *Pythium* sp., and others. Under dry conditions, these organisms are stimulated by seed exudate to become more active as pathogens. These conditions can be easily reproduced by incorporating osmotic compounds, such as mannitol, at potentials pre-established in the substrate, blotters or other media. In these assays, the fungi are also grown on the modified osmotic substrate to which seeds will be placed.

Application of the water restriction technique, as a means to reproduce a dry condition in vitro, can be explored to investigate the behaviour of storage fungi in relation to seeds. As those microorganisms, mostly species of *Aspergillus* and *Penicillium*, present a strong ability to develop in fairly dry conditions, as illustrated in Table 4, the water restriction technology arises as an useful tool

**Table 4. Minimum values of Relative Humidity required for the development of storage fungi.**

Fungi	Minimum Relative Humidities (%)
<i>Aspergillus halophilicus</i>	65
<i>Aspergillus restrictus</i>	70
<i>Aspergillus glaucus</i>	73
<i>Aspergillus candidus</i>	80
<i>Aspergillus flavus</i>	85
<i>Penicillium</i> sp.	85-90

Source: Christensen and Kaufmann (1965)



**Fig. 3. Effects of inoculum potential of *Bipolaris sorokiniana* on the performance of fungicide treated (T) and untreated (NT) seeds of wheat. On horizontal axis: mean percentages of the surface area of the seed taken over by growth of the fungus and one additional control (Saprop.) meaning seeds exhibiting only saprophytes fungi (Celano *et al*, 2002).**

**V - Recovering healthy seeds in a contaminated lot**

Considering that pre-conditioned seeds do not germinate during the osmotic treatment and they can be dried afterwards and stored for a reasonable period of time, healthy looking seeds can be picked out singly in the middle of infected or contaminated seeds of a same lot. For valuable seed batches in which infection levels are high, e.g. 70-80%, the use of the water restriction technique may be helpful to recover or to save 20-30% of healthy seeds. Although seed treatment in those cases can be successful, the use of water restriction may be an alternative approach with some advantages. An outline of how healthy seeds may be selected from a heavily contaminated sample of seeds, by means the osmotic technique is presented in Figure 4.

**VI - Final remarks**

The water restriction technique may be also applied successfully in many other areas of Seed Pathology; the use depends on the objective of each case. From this paper, the integration between seed pathologists and seed technologists becomes more and more necessary to solve several problems in the field of Seed Science and Technology. As these professionals come together to explore their experience and expertise in integrated efforts, many aspects can be learned and then enourmous and rapid progress can be made. ■

**ACKNOWLEDGEMENTS**

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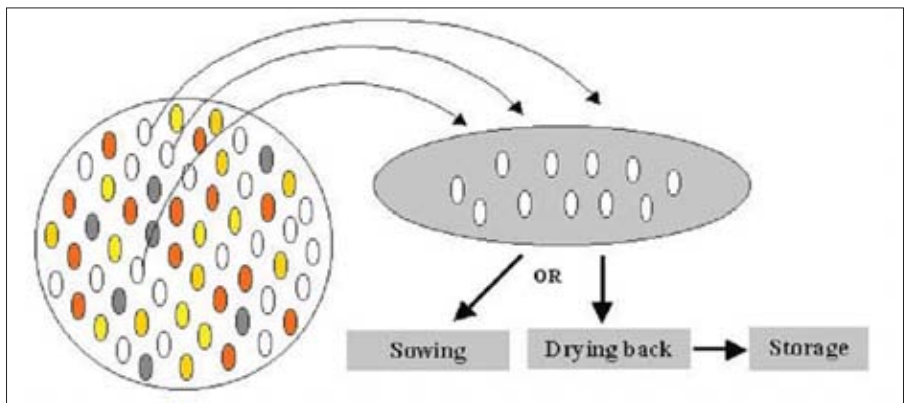
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**Fig. 4. Outline of picking out healthy seeds from a heavily contaminated seed sample by means the osmotic technique**

# Varietal difference between artificial and natural infection of *Ustilago avenae*. The importance of size of kernels and main location of infection

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## Summary

Swedish Seed Testing and Certification Institute is testing and certifying seeds of different species and potatoes. In Sweden all seed lots of cereals are tested for seed health. Seed lots of pre-basic, basic and certified seed of 1st generation of oat are since 1995 tested for *Ustilago avenae* to determine whether the seed have to be chemically treated or not. To obtain a better understanding of the disease we performed three experiments.

The aim of the first experiment was to determine if it is relevant with different limits of number of spores for different varieties.

The varieties were ranked according to susceptibility after artificial infection and natural infection. For some varieties the ranking of susceptibility differed between artificial and natural infections.

The second experiment showed that if seed should be analysed before being graded, a laboratory grading should be done before analyse.

The third experiment showed that the method used in Sweden today is not sufficient. We do not properly detect the spores mainly causing the infection. Although this is the best alternative for detection we have today.

## Introduction

Oat varieties differ in susceptibility to *Ustilago avenae*. Is it relevant to group the varieties according to susceptibility and allow different tolerance regarding number of spores of *Ustilago avenae*?

Sampson (1928) noted that the extent to which oat varieties open their pales is an important factor, which determines the intensity of a smut attack of the crop. It is not improbable that a variety, which has high infection figures when tested under artificial experimental conditions, is among the most susceptible varieties under natural infection. Johnston (1927) found that some varieties of

oats escape infection through protection of their hulls, while others have protoplasmic resistance.

Therefore we made an experiment ranking varieties according to susceptibility when artificially infected compared with natural infection.

When analysing different parts of seed lots Magyarosi (1996) showed that small kernels have a higher number of spores per gram than bigger seeds. Analyses done at our institute showed the same results

Samples of not graded seed are often sent in for analysis of loose smut. Should these samples be graded before analyse with the risk of spreading spores from one sample to another?

In older literature both spores inside and outside of glumes and resting mycelium infecting husk and pericarp are mentioned as infection sources of loose smut on oats, Zade (1924), Sampson (1928), Kolk (1930), Butler and Jones (1949), Malone and Muskett (1964), Neergaard (1977), McEwan (1997). Mac Ewan (1997) did not get any clear evidence for the presence of resting mycelium. In order to determine if a seed lot shall be chemically treated or not it is important to know where the most important source for the infection is situated. To do this you need an accurate analyse of the disease.

## Material and methods

*Experiment 1: Is there any difference between artificial and natural infection of loose smut in oats of different varieties?*

During the summer 1995 a field experiment was done including 37 different oats varieties. The kernels were dehulled by machine to be able to get a higher infection level. This is the procedure that the breeding-company Svalöf-Weibull use when testing varieties for resistance to *Ustilago avenae*. The disadvan-

tage with dehulling is that varieties differ in ability to get rid of the hulls. The varieties have varying amounts of remaining hulls after dehulling. Varieties with more remaining hulls are more difficult to get infected due to the protection from the hulls.

Mixing 180 g of oats with 0.35 g spores of *Ustilago avenae* and 500 ml of water made the artificial infection. The mixture was shaken and left for ½ hour. Afterwards the kernels were dried in room temperature.

37 different samples of 180 g of oats for each variety infected with *Ustilago avenae* were sown on 29th of April. To check if the seedlots also were naturally infected 180 g of not artificially infected seedlots were sown beside the infected plot as a control. Each plot was 8 meter long with six rows.

Every second plot was an infected plot. There were no replicates.

Dehulled kernels also have a lower germination capacity. Therefore, the number of plants were determined by counting two times 1 meter in each plot after emerging, but before tilting started. The numbers of infected plants were recalculated to a number of 1600 plants per plot.

During summer their neighbour infected plots naturally infected the plots sown with not infected seed as the spores were blown around by wind in the experimental field. These naturally infected plots were harvested in autumn.

180 g of the naturally infected seed was sown 1996 with the same plot size as previous year. The seeds were sown 19th of April. The numbers of plants were estimated in the same way as 1995 but the variation this year was less. The number of plants per plot was 3500 - 4000.

1996 a new experiment was performed. This time the kernels were not dehulled before the infection was made in the same way as the experiment 1995. The seed was sown 19th of April. The plot size and the examination

### Healthy seeds, the basis for sustainable farming

were also made in the same way as 1995. The numbers of plants were approximately 3400 per plot.

Also 1996 control plots of not infected seeds were sown beside the infected seed of the same seedlot. This summer these plots were naturally infected not only by the 1996 artificially infected plots but also of the 1995 naturally infected plots grown on the same field.

The seeds being naturally infected 1996, were sown 10th of April 1997. The number of plants per plot 1997 was approximately 4000 plants per plot.

**Experiment 2:** *How important is the size of kernels for the percentage of infection of loose smut in field?*

Four plots of different varieties naturally infected 1995 were chosen. Small sized kernels passing a 2.0 mm slot hole screen but not a 1.8 mm were sown as well as normal sized kernels. 180 g of small respectively normal sized kernels was sown.

1997 four naturally infected seedlots from 1996 years experiment were chosen. The four varieties were Freja, Martin, Svea and Veli. We usually sow 180 g of kernels per plot for postcontrol. The thousand-kernel weight for oats is approximately 37 gram.  $180/37 = 4,864 \times 1000 = 4864$ .

The seed lots were graded in the same way as 1996. From the fraction of small kernels, normally rejected in commercial seedlots. 4864 kernels were counted with Numigral seed counter. In the same way 4864 normal kernels were counted. The plots were sown in the same way as in experiment 1 with no replicates.

**Experiment 3.** *Where is the most important infection source situated?*

Analysis of seeds for certification in Sweden is done with the washing method. Hence the aim with the experiment was to see how important the spores washed away from the seed and detected in the analysis, are for the infection, compared with the spores remaining on the seed after washing. Does the infection level decrease if the seeds are dehulled?

In Sweden the washing method is used for analysis of loose smut on oats. 75 g kernels

are mixed with 100 ml of water and a few drops of detergent, added to reduce the surface tension. The flask is stirred and 2 ml of the suspension is transferred to a test-tube. After nine minutes of centrifugation with 1500 rotations per minutes, the water is thrown away and the sediment attached to the test-tube is mixed with 0.12 ml of a mixture of 2/3 glycerine and 1/3 ethanol (96 %). A smaller amount is transferred to a counting chamber with a fixed volume and the spores are counted under microscope. As we know the volume analysed and the weight of the kernels it is possible to get the result as spores per gram. To get the results as number of spores per gram kernel the number of spores found is multiplied with a factor of 44.

The decision whether the seed lot has to be treated with chemicals is made due to the number of spores found.

The seed lot used in the experiment was a naturally infected seedlot of the variety Kapp from Norway. When grown under field condition in 1996, 8.3% of the plants were infected with loose smut.

600 seed were chosen out of the seed lot. 300 seeds were dehulled separately by hand. Half of the dehulled seeds and half of the non-dehulled seeds were washed. The washing was made according to the same principle as the analysis of loose smut on oats. The sample was stirred in flask containing water with a few drops of detergent and then dried in room temperature.

The oats were grown in peat-mould soil in greenhouse where it was possible to regulate the temperature. The temperature was set up to get conditions favouring the mycelia of *Ustilago avenae* according to the principle that the breeding company Svalöv-Weibull uses for test of resistance to loose smut on oats in their breeding material. Temperature 20-22 °C from planting until the first leaf was developed. Then it was decreased to 16°C during daytime and 14°C during night. Artificial light was added during 18 hours each day. Each plant was pulled out and examined when the plants had grown to that stage that all the panicles were well developed.

There were four treatments in the experiment. In each treatment 150 seeds were sown. There were no replications.

Treatment A. No treatment  
Treatment B. Washed seed with glumes  
Treatment C. Dehulled seed  
Treatment D. Dehulled and washed seed

### Results

**Experiment 1:** *Is there any difference between artificial and natural infection of loose smut in oats of different varieties?*

The seed lots used in the experiment had at maximum two infected plants per plot in the not infected seeds used as control

The variety Martin, that also in practical cultivation has shown susceptibility of loose smut, got a very high percentage of infected plants after natural infection. When it was artificially infected it was not the most infected variety.

A comparison of varieties ranked in order of susceptibility gave following result.

See Tables 1 and 2.

**Experiment 2:** *How important is the size of kernels for the percentage of infection of loose smut in field?*

See Tables 3 and 4.

**Experiment 3.** *Where is the most important infection source situated?*

Some plants had parts of the panicle infected. There were also plants where one or more of the panicles were infected but also bearing panicles with uninfected kernels.

See Table 5.

### Discussion

**Experiment 1:** *Is there any difference between artificial and natural infection of loose smut in oats of different varieties?*

The results shows that it is not the variety which has the highest percentage of loose smut after artificial infections that also have the highest percentage of infection after natural infection. This indicates that the ability of escaping infection is both due to the protoplasmic resistance and the anatomy of the plant and how easy the spores can come under the glumes. That is also the con-

**Table 1. Ranking of varieties in susceptibility to infection of loose smut, artificial and natural infection 1995 and 1996. Number of plants per plot.**

Variety	Infected plants per plot 1995* Artificially infected	Variety	Harvest postcontrol 1996 infected plants per plot naturally infected 1995
Matilda	6	Matilda	0
Svea	8	Selma	0
Vera	21	Svea	1
Sang	24	Vera	1
Rhiannon	25	Vital	4
Adamo	25	Veli	7
Elin	31	Bellinda	12
Svala	34	Solo	12
Vital	36	Edit	13
Diana	42	Frigg	14
Stork	44	Freja	15
Edit	49	Adamo	16
Solo	54	Stork	16
Sanna	55	Diana	17
Bodil	62	Doris	17
Selma	65	Petra	20
Freja	65	Silvano	20
Veli	69	Elin	24
Puhti	73	Rhiannon	25
Petra	83	Sanna	25
Frigg	86	Sang	25
Silvano	90	Galopp	37
Pol	92	Bodil	40
Doris	92	Svala	47
Martin	92	Puhti	47
Galopp	97	Pol	79
Bellinda	168	Martin	200

**Table 2. Ranking of varieties in susceptibility to infection of loose smut, artificial and natural infection 1996 and 1997. Number of plants per plot.**

Variety	Infected plants per plot 1996 Artificially infected	Variety	Harvest postcontrol 1997 infected plants per plot naturally infected 1996
Hirondel	0	Hirondel	0
Svea	52	Vital	1
Doris	86	Petra	2
Adamo	101	Adamo	3
Martin	118	Freja	3
Alfred	144	Alfred	4
Vital	156	Svea	5
Sanna	172	Doris	6
Sang	207	Valiant	7
Petra	217	Galopp	10
Veli	242	Vera	11
Vera	245	Sang	12
Freja	250	Sanna	14
Galopp	260	Veli	15
Valiant	356	Silvano	38
Silvano	365	Martin	52

**Table 3. Percentage of infection of loose smut on oats depending of size of kernels**

Variety and grading	Number of infected plants per plot 1996
Freja small kernels	108
Freja normal sized kernels	15
Martin small kernels	appr 450
Martin normal sized kernels	appr 200
Pol small kernels	197
Pol normal sized kernels	79
Svea small kernels	3
Svea normal sized kernels	1

**Table 4. Difference in percentage of infection for the same number of kernels with different size.**

Variety and grading	Weight of kernels sown per plot	Number of infected plants per plot 1997
Veli small kernels	100g	39
Veli normal sized kernels	161g	7
Martin small kernels	105g	279
Martin normal sized kernels	162g	62
Svea small kernels	109g	30
Svea normal sized kernels	161g	4
Freja small kernels	102g	31
Freja normal sized kernels	161g	5

**Table 5. Number of plants with one or more infected panicles of loose smut on oats.**

	Treatment A	Treatment B	Treatment C	Treatment D
Number of plants	148	141	144	132
Number of partly or totally infected plants	47	36	12	1
Percentage of infection	31.8	25.5	8.3	0.8

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clusion of Johnston (1927). A variety with a high percentage of loose smut after artificial infection but a lower after natural infection has probably an anatomy that makes it more difficult for the spores to be able to approach under the glumes. Also Sampson (1928) wrote, "It is not improbable that a variety which gives high infection figures when tested under special experimental conditions will not necessarily fall among the most susceptible varieties when natural infection is in question".

Also barley shows difference in susceptibility to loose smut (*Ustilago nuda* (Jens.) Rostr.) due to difference in tendency to open flowering (Pedersen 1965).

The variety Selma in the experiment 1996 and Valiant in experiment 1997 shows this pattern and probably both have the anatomy that prevents the spores to get into the flower. With many new varieties entering the market it is complicated to have different tolerances for different varieties.

**Experiment 2:** *How important is the size of kernels for the percentage of infection of loose smut in field?*

The experiment showed that small kernels give rise to a higher percentage of infection. If seed should be analysed before being graded, a laboratory grading should be done before analysis. Otherwise it is easy to get a too high result of the analysis and a misleading advice for decision of chemical treatment. In Sweden it is common to use this type of analysis for the decision of chemical treatment of graded seed without making any new analysis of the graded material.

**Experiment 3.** *Where is the most important infection source situated?*

There is a big difference in percentage of infected plants between the dehulled and not dehulled kernels.

This indicates that the main source of infection is situated underneath the glumes. Treatment B and D, being washed, shows a lower infection rate than treatment A and C, not being washed. This indicates that the spores that were washed away have some importance for the level of infection.

There is no clear answer whether the infection source is resting mycelium or chlamydospores, but the fact that washing and reducing the amounts of spores also reduce the percentage of infection, points to that the spores have consequences for the survival of the infection. The low percentage of treatment D indicates that mycelium attached to the surface of the kernel or inside the embryo is not responsible for the infection.

These results support the results of MacEwan (1997) that she could not get any clear evidence of the presence of resting mycelia. If mycelia still are responsible for at least some of the infection, it must be attached to the hull, the results also given by Samson (1928). ■

#### Acknowledgements

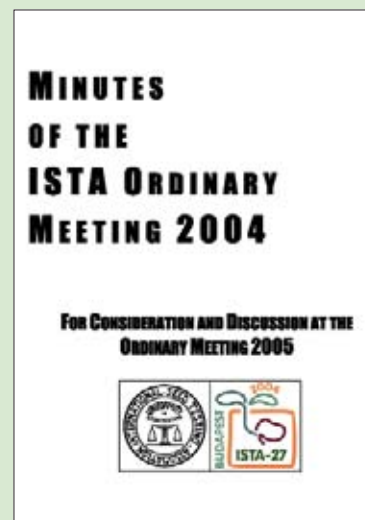
I want to thank colleges of the Institute for helpful assistance with the experiments and comments of the manuscript.

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## Minutes of the ISTA Ordinary Meeting 2004

Cited and Published  
By the ISTA Secretariat



This document summarizes and concludes the discussions and decisions of the ISTA Ordinary Meeting 2004 in Budapest, Hungary, May 20 and 21. The full spoken text of the meeting is not quoted in these Minutes. However, the whole Ordinary Meeting has been recorded on tape, and is at the disposal of any interested person.

These minutes contain a complete list of all participants of the meeting. All documents and presentations mentioned in these minutes are available from the ISTA Secretariat.

These 'Minutes of the ISTA Ordinary Meeting 2004' are available from the ISTA Secretariat as from October 2004. Price\*: Swiss Francs CHF 70.- (approximately EUR 45.- / USD 55.-) or as free download from the ISTA Website.

\*Note: The document will be distributed electronically to all ISTA Members, Designated Authorities and all participants of the 27<sup>th</sup> ISTA Congress 2004 as a free service. Any member requiring a printed copy, please contact the Secretariat.SS

See more details on upcoming ISTA Meetings on page 6.



# A Comprehensive Worldwide Questionnaire on Flower Seed Testing



By **Lea Mazor**, ISTA Flower Seed Committee, Former Vice-chair, ARO, Volcani Center, Israel, e-mail: leamazor@volcani.agri.gov.il

## Introduction

In 1998, members of The Flower Seed Committee (FSC) at the Pretoria Congress decided to send a questionnaire to seed testing laboratories worldwide.

The questionnaire was aimed to determine the needs and requirements of the various stations and the seed industry, as well as to identify the problems they encounter.

An extensive questionnaire was distributed in 1999 to all ISTA stations, AOSA/SCST laboratories, official and non-official laboratories, ISF Secretariat, and several dozen of seed companies worldwide.

The questionnaire provided volume of database from all over the world. A total of 97 laboratories and companies from 40 countries responded, including 56 laboratories which were engaged in testing flower species.

The data obtained from the 56 stations/laboratories dealing with flower species were organized, sorted, analyzed and classified into several categories.

## Results and conclusions

The type and number of laboratories which test flower seeds and responded to the questionnaire is shown in Table 1. The classification into the various laboratory types is based on the list of ISTA Accredited labs. Over 50% of the laboratories were ISTA governmental, confirming that flower seed testing is now commonly used in ISTA labs.

**Table 1: Types of labs which test flower seeds**

Laboratory Type	No. of labs
ISTA government	30
ISTA private companies (incl. 1 SCST lab)	3
AOSA/SCST (incl. US seed companies)	10
Seed Companies (ISF)	13
<b>Total</b>	<b>56</b>

## The questionnaire distributed was divided into 6 parts

**Part 1:** Data about the type of tests performed and number of flower seed samples. This included general query on objectives of the FSC, the principal problems encountered, kind of information needed, and priorities for activities or issues.

**Part 2:** Species within the ISTA Rules to be revised.

**Part 3:** New species proposed for adoption to the ISTA Rules.

**Part 4:** List of 20 globally important species and type of tests performed (purity, germination, dormancy breaking, seed vigor, seed-borne diseases etc).

**Part 5:** List of 10 top most frequently tested species.

**Part 6:** Names of volunteers willing to assist FSC.

## The main findings and recommendations derived from the questionnaire are summarized therein

### Part 1: General questions

#### Question 1: Determine the most important objective of the FSC

Answers obtained in order of priority:

1. To introduce new species into ISTA Rules, including standardizing their testing procedures for purity, germination (dormancy breaking), tetrazolium etc. Also, updating seed testing methods and evaluation of species within the ISTA Rules.
2. To prepare working sheets on important flower species (Handbook).
3. To deal with and examine problems

encountered by members.

4. To develop testing methods that would predict percentage of useable plants and field performance.

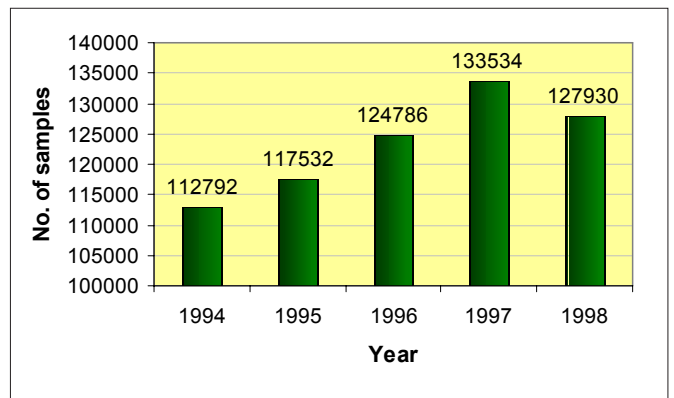
#### Question 2: Number of flower seed samples tested by 56 labs

During the years 1994-9, the number of samples tested annually by the 56 labs, varied from about 110,000 to 135,000 (Figure 1), averaging 2450 samples for a lab per year. Generally, it seems that this amount has increased during the years, though for specific laboratories it has either increased or decreased.

On the basis of the number of flower seed samples tested per year, the labs were divided into four categories (Table 2). As expected, the greatest amount of samples (>10,000) was tested by some seed companies. Of the ISTA labs, only 5 tested >1,000 flower seed samples per year, while the remaining tested 10 to 1,000 samples per year.

#### Question 3: Frequency of main tests conducted annually on flower seeds

As expected, the germination test was conducted most frequently (90%) by the majority of the seed labs, while the purity test was carried out in 55% and tetrazolium only in 10% of the samples (Table 3).



**Figure 1: Total number of flower seed samples tested annually, by 56 labs**

**Table 2: Number of flower seed samples tested by the labs (per year)**

No. of samples	>10,000	1,001-10,000	101-1,000	10-100
No. of labs	3	15	22	16
Type of labs	seed companies	AOSA/SCST Seed companies + 4 ISTA Labs		

**Table 3: Main tests conducted on flower seeds (frequency of each test)**

\*Frequency among labs that conducted this test

Kind of test	Average (%) frequency*
Germination	90
Purity	55
1000 Seed wt.	48
Moisture	41
Tetrazolium	10
Vigour	13
SBD-Fungi	32
SBD-Bacteria	3
SBD-Viruses	5

### Question 4: Principal problems encountered

In testing **purity** the problems encountered in order of priority were:

- I. Excessive mass of 'inert matter' and difficulty in distinguishing between seeds versus flower plant parts when similar in appearance.
- II. Identification of 'other/weed seed'.
- III. Identification of flower species in mixture.
- IV. Pure Seed Definition (PSD) for flower species.

In testing **germination**, the leading problem was to determine the type of dormancy and methods for breaking it. The following problems were encountered as well:

- I. Germination conditions and evaluation of flower species in a mixture.
- II. Inconsistency between germination results in the lab versus field conditions.
- III. Predicting usable plants in soil.
- IV. Lack of testing methods for wild species and species not included in the Rules.
- V. Empty 'pure seeds', resulting in inaccurate evaluation of germination percentage.

### Question 5:

#### Information required for flower seed testing

Purity: in order of priority

- I. Pure Seed Definition (PSD) for flower species.
- II. Weight of seed samples for unlisted species.

III. Identification of 'inert matter' and 'other seeds'.

IV. Identification of flower seed species.

V. References- specimen for flower seed collection.

VI. A Handbook.

Germination: in order of priority

I. Germination conditions (temperature, substrate, lighting, moisture, test duration) and evaluation of seedlings, including drawings.

II. Methods for dormancy breaking.

III. A handbook.

IV. Methods other than those approved in the Rules which have been successfully used with problematic species.

Other tests requested were the tetrazolium test, seed moisture, and methodology for testing seed-borne pathogens.

ID book (Handbook) on flower seed, in color, with common and botanical names was a common requirement.

### Question 6: Priorities for necessary activities of FSC

In general, several labs requested germination methods for flower species not listed in ISTA Rules, as well as methods for breaking dormancy. Other labs were interested in standardization for measuring speed and uniformity of germination.

Several labs expressed the need for a workshop. This request was fully met in May 2003, when the first workshop was held in Budapest.

A comprehensive Handbook (consisting of integrated working sheets for each species) and a wider range of species than is currently available in ISTA, would be welcomed by most labs.

In this respect, the committee fairly fulfilled the laboratories' requests, as these last two items were carried out during 2000-2004.

### Part 2: Flower species within the ISTA Rules that need revision

In general, labs are satisfied with the current methods of ISTA Rules. A few proposals were submitted to the FSC, such as reducing the duration of germination tests and reducing the 'working sample' for several species. A suggestion was also made to reduce the number of permissible germination methods for a given species. On the other hand, some proposed to add soil or/and compost (soil-less mixture) as a primary substrate for flowers, as presently practiced in the private sector.

In conclusion, the labs indicated that there is no need to revise the ISTA Rules. Thus, the common view that the testing methods are not up to date was found incorrect.

### Part 3: New flower species to be adopted into the ISTA Rules

Fifty one new species were proposed to be included into the ISTA Rules, some of which are not flower species. Among the flower species are *Eustoma russellianum*, *Mesembryanthemum tricolor*, *Centaurea jacea*, and *Solidago virgaurea*. The labs provided valuable testing information for these new species, such as purity, germination and dormancy breaking methods, as well as the lab expertise and readiness to provide additional information.

### Part 4: Data on the 20 globally most important flower species according to the Survey on testing activities of ISTA stations in 1991

A list of 20 most frequently tested flower species, according to the Survey on testing activities of ISTA stations in 1991, was attached to the questionnaire.

Members were asked to fill in enclosed Tables in order to provide recent information on these species.

The data presented in Table 4a and 4b show figures of purity, germination, dormancy breaking, tetrazolium, seed vigor and seed-borne disease tests, based on an average of the last 5 years. The labs supplied records on methods based on ISTA and AOSA Rules, as well as in-house methods.

The data collected is unique and important. It illustrates the type of tests that are actually being done by the labs, in addition to purity and germination, and the number of labs conducting each test. For example, the data provides information as to which labs perform tetrazolium test of *Petunia* spp. and which have the expertise in testing seed borne-disease of *Callistephus chinensis*.

This information should be known to all FSC members and be incorporated into the flower seed Handbook. A copy of the methods other than those of ISTA/AOSA is available.

### Part 5: Top 10 species most frequently tested

Each laboratory was asked to specify the 10 most important flower species they test and provide any available information, however limited, in the same pattern as in Part 4.

The data obtained show the list of most frequently tested species for each type of test, separately (Table 5a and 5b). The most fre-

quent species in this questionnaire were different from those in the 1991 Survey. This may reflect the fact that, unlike the 1991 Survey, the present one also included seed companies, private labs and AOSA/SCST. The most frequent species are the following: *Petunia* spp., *Pelargonium* spp., *Impatiens* spp. and *Cyclamen* spp. Notably, the last 3 species were not included among the 20 important species in the 1991 Survey. Moreover, information on 8 additional new species not yet covered by the ISTA Rules was obtained.

Therefore, this part illustrates the importance of organizing such a survey periodically.

In 2003, a survey conducted by Mrs. Zita Ripka, the ISTA FSC chairperson, was sent to all ISTA member labs. The most frequently tested species were found similar in both surveys. However, in the 1999 questionnaire, the number of samples was much higher, reflecting the participation of flower company labs. The new survey lends further support to the validity of the results described here.

**Part 6: New volunteers to assist FSC**

A list of volunteers was proposed to the chairperson of FSC. Some of them are

experts in numerous flower species and can contribute significantly to FSC.

**Conclusions**

1. The complete report provides valuable information on flower seed testing. The report enables FSC to improve services to the labs and, hopefully, will contribute to the FSC future programs.

2. The strategy and priorities of FSC regarding the preparation of working sheets and the initiative to publish a Handbook is indeed the correct approach. The replies emphasize the need to include additional flower species as well as additional information regarding other tests such as dormancy breaking, tetrazolium, seed-borne diseases, as well as references, guidance and recommendations. The report provides important data for the Handbook.

3. A common request was to include new species into the ISTA Rules. A list of 51 species is presented with limited, not yet sufficient data.

4. The current ISTA methods for testing purity, 1000 seed wt. and germination appears to be sufficient. In general, there is no need to review the existing ISTA Rules.

5. The first flower testing workshop was held a year ago in Budapest, fulfilling the general request of FSC members.

**Recommendations**

1. A complete report of the 1999 questionnaire including detailed replies by the labs, as well as analysis of the results and data on testing methods (ISTA, AOSA and in-house) is available and has been submitted to the FSC. It is recommended that this report be consulted for each working sheet of the Handbook.

2. To insert in the ISTA FSC Web site a special file that includes in-house methods for the testing of flower species. This site will not be under the ISTA responsibility, but will offer informal information and links.

3. To form a new working group that will assist in introducing new flower species into the ISTA Rules, according to the 'ISTA Handbook of Method Validation for Seed Testing'.

4. Compost-soil is an important substrate in flower seed industry. The Germination working group may investigate this topic for the benefit of seed testing labs.

5. To participate in the new ISTA 'Mixture Working Group' for preparation of the procedure: 'How to test and report of flower species mixture'.

**Acknowledgments**

I would like to thank Mrs. Henriette Schmiermann and Mrs. Sharon Davidson for their assistance. ■

**Table 4a: List of 20 globally important flower species according to the 1991 Survey**

Species	Purity		Germination		Dormancy breaking	
	No. of samples	No. of labs	No. of samples	No. of labs	No. of samples	No. of labs
<i>Petunia X hybrida</i>	61	1	<u>12578</u>	34	345	11
<i>Viola tricolour</i>	377	19	5747	35	<u>528</u>	13
<i>Antirrhinum majus</i>	240	18	4745	36	301	14
<i>Tagetes</i> spp.	223	12	4120	29	198	4
<i>Callistephus chinensis</i>	<u>1051</u>	19	2560	34	43	3

**Table 4b: List of 20 globally important flower species according to the 1991 Survey**

Species	Tetrazolium		Vigour		SBD	
	No. of samples	No. of labs	No. of samples	No. of labs	No. of samples	No. of labs
<i>Petunia X hybrida</i>	<u>60</u>	1	<u>275</u>	1	21	3
<i>Viola tricolour</i>	54	2	210	2	<u>67</u>	3
<i>Antirrhinum majus</i>	26	2	100	1	8	3
<i>Tagetes</i> spp.	25	1	64	1	1	1
<i>Callistephus chinensis</i>	11	2	20	1	51	2

**Table 5a: Top 10 species most frequently tested**

Species	Germination	Dormancy breaking	Purity
	No. of samples	No. of samples	No. of samples
<i>Petunia X hybrida</i> +P. spp.	<u>12757</u>	390	428
<i>Viola tricolour</i> +V. spp.	9551	<u>693</u>	777
<i>Pelargonium</i> spp.	6415	-	1103
<i>Tagetes</i> spp.	6384	106	562
<i>Callistephus chinensis</i>	2819	43	<u>1993</u>
<i>Salvia</i> spp.	1078	157	191

**Table 5b: Top 10 species most frequently tested**

Species	Tetrazolium		Vigour		SBD	
	No. of samples	No. of labs	No. of samples	No. of labs	No. of samples	No. of labs
<i>Petunia X hybrida</i> +P. spp.	-	-	275	1	-	-
<i>Viola tricolour</i> +V. spp.	-	-	212	-	<u>66</u>	2
<i>Pelargonium</i> spp.	<u>15</u>	1	<u>760</u>	1	-	-
<i>Tagetes</i> spp.	-	-	-	-	23	4
<i>Callistephus chinensis</i>	-	-	-	-	51	2
<i>Salvia</i> spp.	-	-	279	1	4	1

# ISTA Rules Update

## An Interview with Steve Jones, Chair of ISTA Rules Committee

### About Steve

Steve has been Chair of the ISTA Rules Committee and a Designated ISTA Member since 2000. From 1988-1997 Steve worked as a seed physiologist at the Official Seed Testing Laboratory for Trees and Shrubs at Alice Holt, Farnham, UK, with Peter Gosling. This is also where Steve first came across the work of ISTA attending the ISTA Forestry Tour of the UK, which was organised by Peter Gosling as the then Chair of the Forest Tree and Shrub Committee. Steve is now Head of the ISTA Accredited Laboratory GBDL01 based at NIAB, Cambridge, UK and was elected to the ISTA Executive Committee in 2004.

### About NIAB

NIAB is a not-for-profit organisation and has been involved with ISTA since ISTA's formation in 1924. The Official Seed Testing Station (OSTS) for England and Wales has been at Cambridge since 1921. The OSTs laboratory provides technical advice to the UK government, and seed trade and currently has a staff of 16, including 13 fully qualified seed analysts. It also provides seed sampler and seed analyst training; and monitors the 32 or more licensed seed testing stations in England on behalf of Defra (Department of Environment Food and Rural Affairs).

### Aims of the article

In this article Steve explains how he sees his role as Chair of the Rules Committee, the Rules Committee structure, recent changes to the International Rules for Seed Testing and the plans for the future.

As Chair of the ISTA Rules Committee the main role is as Editor of the International Rules for Seed Testing: ISTA Rules for short. This role is very much a team effort with help and support from the Rules Committee members and the ISTA Secretariat, as well as the membership who help spot anomalies in the Rules. The ISTA Rules are very much a working tool for the members so they need to be accurate, easy to use and helpful. The Rules are currently available in English and are being translated into French, German, Italian, Danish and Chinese. When the text is being translated that is often when questions

arise and any errors are spotted. Although it is a team effort, as Chair of the Rules Committee, Steve does feel personally responsible when errors are found.

The Rules Committee is an important check on the rules proposals as well as being able to contribute to discussion on any difficult problems. The Rules Committee structure is unique within ISTA. The Rules Committee currently consists of the chairs of all the other ISTA Committees and Task Forces, as well as the Honorary ISTA President, and a representative from the Editorial Board.

### The ISTA Rules Committee Members are as follows:

- Dr. Steve Jones, United Kingdom
- Dr. Anne Bülow-Olsen, Denmark
- Mrs. Valerie Cockerell, United Kingdom
- Mr. Ronald Don, United Kingdom
- Dr. Sylvain Grégoire, France
- Dr. Harm Huttinga, Netherlands
- Mrs. Stefanie Krämer, Germany
- Prof. Dr. Michael Kruse, Germany
- Prof. Dr. Norbert Leist, Germany
- Prof. Dr. Attilio Lovato, Italy
- Dr. Maria-Rosaria Mannino, France
- Dr. Günter Müller, Germany
- Prof. Dr. David John Mycock, South Africa
- Mr. J. Harry Nijënstein, Netherlands
- Dr. Alison Powell, United Kingdom
- Mrs. Zdenka Procházková, Czech Republic
- Mrs. Zita Ripka, Hungary
- Dr. John H. Wiersema, United States

The fact that we have moved to annual meetings is good for ISTA in being able to respond to changing needs quicker but it now means we need to produce and collate any rules proposals annually. This makes more work for the Technical Committees as it is the Technical Committees that need to produce the rules proposals, it is then down to the Rules Chair to collate all the proposals, look for cross-chapter problems or related changes and circulate them to get feedback from members of the Rules Committee. The



preparation of documents for printing and circulation to the ISTA Membership comes next and this is where the ISTA Secretariat comes in.

The Rules Chair also acts as spokesperson for the Rules, so is the one who presents and explains the proposals at the voting meetings. The ISTA Secretariat by editing and recording the voting decisions on the Rules proposal again supports the voting process. Then the hard work of amending the Rules and getting the changes out to the membership starts. The first version is checked by the Chair and then by circulation to the other committee members. The preparation of the printed versions used to be done by Jim Sheppard but is now done within the Secretariat, usually by Bettina Kahlert and her colleagues, while Bettina is on maternity leave. The aim is to provide the printers with a 'ready to go' version to prevent the need to proof read again after submission to the printers.

This explains the 'normal' routine for the Rules proposals, voting and amendments but at the ISTA Meeting in Budapest the Executive Committee accepted the idea of amalgamating some of the Chapters in the Rules over the next few years. Although it was agreed the loose-leaf version of the International Rules for Seed Testing was an extremely good, user-friendly format it was thought they could be further improved by merging the Rules and Annexes to make them more readable and easy to use. Not all Chapters may need this but the Bulking and Sampling Committee (BSC) thought theirs did, so they have taken on this task as a 'pilot project'. Although this can be thought of as a pilot project it still needs to fit into the 'normal' routine for the operation and modification to the Rules. To help this happen the process for the 'pilot' amalgamation is as follows:

1. Chapter 2 will be amalgamated by the BSC and approved by the Rules Committee and the scrutiny team of the Rules Committee. The result should be an amalgamation of text from the Rules and Annexes and deletion of any superfluous text. A special scrutiny team has been formed as consisting of ex-ISTA Executive Committee members to help validate this process. This team consists of Doug Ashton, Kevin Boyce and Simon Cooper.

2. The ECOM will be informed in January 2005, or sooner, when a suitable editorial amalgamation is ready.

3. The amalgamated chapter will be circulated to all ISTA Members and stakeholders in January 2005, or sooner. This is not a voting matter if it is editorial changes only. The members will receive the statement that the Rules Committee has approved this amalgamation and that it is of purely editorial nature.

4. The Bulking and Sampling Committee will elaborate Rules Changes on basis of the amalgamated Rules Chapter, which will be brought to the attention of the ISTA Members as normal in the ISTA Rules Proposals 2005. This document will be sent out in February 2005 to all ISTA Members.

5. In April 2005 the ISTA voting delegates will vote on the ISTA Rules Proposals in the Ordinary Meeting in Bangkok, Thailand, as usual.

6. Depending on the outcome of the votes, the proposed Rules Changes will be incorporated into the Rules as normal and in the case of Chapter 2 into the amalgamated chapter.

7. The amalgamated Chapter 2 including the adopted Rules Changes will be printed and will come into force on January 1, 2006.

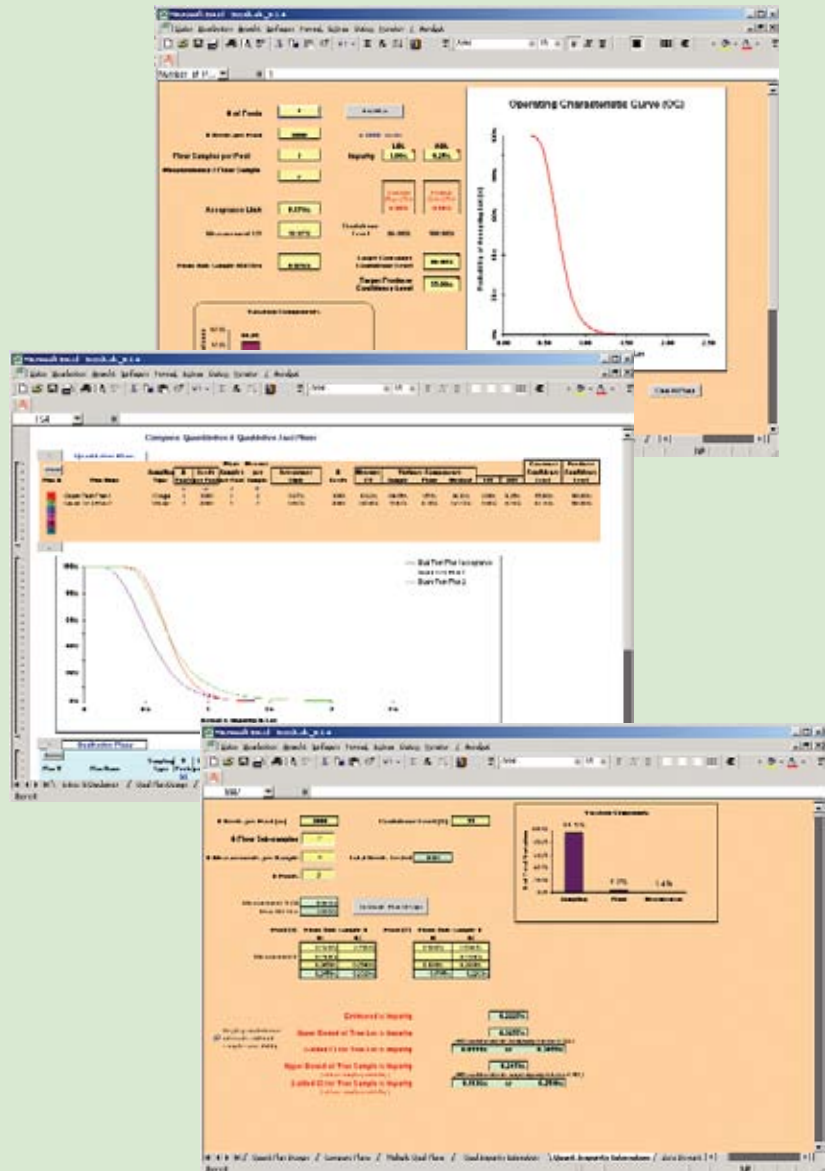
So as we take another step forward as usual the participation and involvement of the ISTA membership is essential and very welcome. If you have any things you want to raise or comments to make on any issues related to the Rules please feel free to contact the ISTA Secretariat at [ista.office@ista.ch](mailto:ista.office@ista.ch) and/or Steve at [steve.jones@niab.com](mailto:steve.jones@niab.com).

**Acknowledgements**

Steve thanks Defra and NIAB for supporting his work for ISTA. He also thanks colleagues at NIAB, the OSTs for Scotland, Jim Sheppard from the CFIA (Canadian Food Inspection Agency) Seed Laboratory in Ottawa, Patricia Raubo and Bettina Kahlert from ISTA for all their help in making the job of Chair of the Rules Committee that much easier. Not least he would like to acknowledge the friendly and positive attitudes towards the Rules, and the Rules Chair, taken during the voting meetings by the ISTA Membership as a whole. ■

# Seedcalc6 spreadsheet application is now available on the ISTA Website

The Seedcalc6 MS Excel® spreadsheet application is now available for free download from the ISTA Website. Seedcalc6 is written for Windows 2000 and XP. All of the capabilities of previous versions of Seedcalc (i.e., Seedcalc3 and Seedcalc5) using a qualitative assay are still available in Seedcalc6. Seedcalc6 is enhanced with the ability to design testing plans and evaluate results from seed testing when a quantitative assay is used such as Real-time PCR. These enhancements to Seedcalc6 are described in the paper entitled "Testing for adventitious presence of transgenic material in conventional seed or grain lots using quantitative laboratory methods: a new statistical approach and its implementation" which has been submitted for publication to The Journal of Agriculture and Food Chemistry. Seedcalc can also be used to design testing plans for traditional seed measurements such as germination and purity.



[www.seedtest.org](http://www.seedtest.org)

# 1<sup>st</sup> ISTA Workshop on Statistical Aspects of GMO Detection - Europe

Toulouse, France, April 1 - 2, 2004

By Sylvain Grégoire, ISTA Statistics Committee Chair

GEVES, France, e-mail: sylvain.gregoire@geves.fr



The workshop organised by ISTA was held in Aussone (France), hosted by Pioneer Genetics. Jean-Louis Laffont, Sylvain Grégoire and Kirk Remund made presentations on 10 different topics:

- Usually found distributions
- Statistical tests
- Uncertainty
- Designing testing plans
- Robustness
- Regulatory example
- GM estimation
- Purity of reference material
- Data checking
- Repeatability

Different free software were also made available and participants used them through exercises. Software, powerpoint presentations, exercises were given on CD-Rom and as a printed version to the participants.

Working sessions were very intensive, allowing only a short visit to the laboratories and an evening meal in the city of Toulouse.

Bettina Kahlert from the ISTA Secretariat, in charge of the GM Proficiency Tests, was present; as well as members of the ISTA GMO Task Force and participants with already great skills in GM detection. Some of the discussions that occurred during this workshop lead to the implementation of new features in Seedcalc software version 6, and some more difficult questions are still under consideration for future improvements.

Due to a limited number of participants, not all persons willing to register were able to attend. Another workshop will be held in St Louis (US) this year. 3 days instead of 2 will allow more time for the computer exercises and a visit. ■

# The ISTA Tetrazolium Workshop

Tunica, US  
June 14 - 16, 2004

By Nancy Vivrette,

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The ISTA Tetrazolium Workshop took place from June 14-16, 2004 in Tunica, Mississippi US. The list of 40 participants reads like a Who's Who in Tetrazolium Testing. Most of the people who trained the speakers were attending the workshop. All crops and sectors of the industry were represented. The staff members of the Mid-West Seed Laboratory are to be commended for putting on a full and informative workshop under perhaps the most unique conditions for a workshop (think blackened nightclub in a casino atmosphere). No one will ever forget this workshop!

The instructors included Augusto Martinelli, Annette Miller, Ron Don, Nancy Vivrette and Maria Alejandra Petinari.

Augusto and Annette described the Tetrazolium Handbooks of ISTA and AOSA, and Augusto described areas of similarity and differences. The main difference is in the length of time for the stain and the resulting degree of stain. Both emphasized the need for continued work, and Annette introduced the AOSA website for adding new methods.

Ron Don presented a series of lectures on 'Reducing the Number of Seed Tested using Tolerance Tables and Sequential Testing'. There was a gasp as he handed out a test on the first morning, but the participants were up to the task. Ron then went right into the preparation of Brassica, and we nearly had a revolt.

Annette brought her collection of tools used in TZ Testing. We used some of the more wicked of these in the preparation of *Tripsacum*, *Buchloe* and *Bouteloua* species. Annette also shared a collection of colored embryos that give TZ analysts trouble.

Nancy Vivrette presented methods to deal with deep dormancy in native species and to stain chlorophyllous embryos. Nancy also

presented examples of damage caused by improper preparation, and a comparison of abnormalities found in germination tests and TZ evaluations of viability in small seeded vegetable seeds.

Augusto and Maria helped us with Glycine and Sorghum. Ron Don helped us evaluate heat damage and coleoptile damage in temperate Cereals. The shock of the meeting was Maria Alejandra Petinari's presentation on Glycine seeds which had been stained with .075% TZ and then were grown out to see the seedlings these staining patterns pro-

duced! This technique has enormous potential value in standardizing our evaluations.

As always, the best part of the workshop was the discussions between the participants. We had some



great arguments. Thank you to all the instructors, the organizers and participants. ■

## ISTA Seed Health Committee Workshop

Novi Sad, Serbia & Montenegro, May 6 - 12, 2004

By Mirjana Milošević, ISTA Member

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ISTA Seed Health Committee Workshop, as an ISTA pre-congress activity, took place from 6-12 May, in Novi Sad, Vojvodina Province, the main agricultural part of Serbia & Montenegro. ISTA, National Laboratory for Seed Testing and Institute of Field and Vegetable Crops were the organizers of this Workshop. Interest in this Workshop was great. There were 47 participants from 20 countries. The topic of this Workshop was fungal diseases on main agricultural crop: maize, wheat, barley, sunflower, soybean and carrot, in this part of Europe.

The scientific program of the Workshop, supplemented with theoretical explanations on seed-borne diseases, was completed in the morning and practical work, visual observation of seedling and pure cultures of fungi under binocular and microscope, in the afternoon.

Invited speakers were members of the ISTA Seed Health Committee. For maize seed-borne diseases, *Fusarium* sp. *Drechslera* sp. it was Prof. Dr. Denis McGee from Iowa State University (USA). Mrs. Valerie Cockerell, Official Seed Testing station, Edinburgh, (UK) was engaged in wheat and barley diseases - *Fusarium* spp., *Pyrenophora* sp., *Tilletia* sp., *Ustilago* sp. Dr. Michael Guenard from GEVES, National d'Essais de Semences, France, gave a lecture on seed-borne diseases on sunflower - *Phomopsis* sp., *Botrytis* sp., *Orobache* sp., *Plasmopara helianthii*, *Rhizopus* sp., *Alternaria* sp. Dr. Krystyna Tylkowska, from Poland, University, Agricultural faculty, seed science department focused on the results relating to organic seed production. She spoke about carrot diseases - *Alternaria* sp.



"Importance of seed-borne *Alternaria* spp. in carrot" (Occurrence of *Alternaria* spp. in carrot seed, disease problems at carrot production chain, including organic farming) which was part of the project: "Safe organic vegetables and vegetable products by reducing risk factors and sources of fungal contaminants throughout the production chain: the carrot - *Alternaria* model".

At the same time speakers from Serbia & Montenegro held lectures too. They focused on the results they obtained relating to seed-borne diseases on above mentioned plant species. General information on seed production and review of main pathogens in Serbia & Montenegro was given by Prof. Dr. Mirjana Milošević, Director of National laboratory for Seed Testing. Prof. Dr. Stevan Maširevic, from Institute of Field and Vegetable Crops, Novi Sad talked on sunflower seed-borne diseases - *Phomopsis* sp., *Botrytis* sp., *Orobache* sp., *Plasmopara helianthii*, *Rhizopus* sp., *Alternaria* sp. Prof. Dr. Stevan Jasic from Institute of Field and Vegetable Crops, Novi Sad gave a lecture on soybean diseases - *Phomopsis* spp. All other speakers were from the Institute of Field and Vegetable Crops. Dr. Bozana Purar informed participants on main seed-borne diseases on maize - *Fusarium* sp., *Drechslera* sp. Dr.

Radivoje Jevtic focused on *Pyrenophora graminearum* and other important diseases on barley and wheat - *Fusarium* spp., *Pyrenophora* sp., *Tilletia* sp., *Ustilago* sp.

The participants were given the "Handbook on seed-borne diseases on maize, wheat, barley, soybean, sunflower and carrot" written by Mirjana Milošević, Stevan Maširevic and Radivoje Jevtic for assistance in their work throughout the Workshop. The lecturers were given a letter of appreciation, and all participants were given certificates of participation in the Workshop.

The atmosphere during the Workshop was very pleasant. Some new acquaintances were made. A ceremonial dinner and music of tamburitza players gave a final touch to a good atmosphere prevailing during the entire Workshop. From the professional side, a visit to the National Laboratory for Seed Testing and Institute of Field and Vegetable Crops in Novi Sad revealed the picture of the host of this Meeting. Visits to museums and monasteries revealed a part of the history of this peaceful Vojvodina plain.

With a good impression and great knowledge acquired during this Workshop, the participants headed on towards the ISTA Congress in Budapest, or to their home. ■

# 4<sup>th</sup> ISTA / FAO Workshop - Electrophoretic & PCR-based Methods for Varietal Verification & GMO Detection Ljubljana, Slovenia, July 10 - 14, 2004

By Jelka Sustar-Vozlic,

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## Introduction

In July 2004 the Agricultural Institute of Slovenia (AIS) hosted the workshop on Electrophoretic and PCR-based Methods for Varietal Verification and GMO Detection. This was already the 4<sup>th</sup> workshop that has been held by ISTA and FAO on this topic. The aim of this workshop was to train the seed testing analysts from laboratories located in the Eastern European region in methods for the verification of species, cultivars and hybrids as well as for qualitative and quantitative GMO detection. Dr. Bettina Kahlert, the head of ISTA Technical Committees Administration, was the coordinator. The number of applications that were received much exceeded the capacities of the laboratory and the budget for funding, which indicates the growing importance for training in the field of variety testing and GMO detection. AIS as the local organizer arranged accommodation, catering and local transportation for the participants and lecturers and prepared facilities at the Institute for the theoretical and practical work.

21 participants from 14 European countries, Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Romania, Estonia, Hungary, Macedonia, Latvia, Poland, Serbia & Montenegro, Slovakia, Slovenia and Turkey attended the workshop in Ljubljana. Unfortunately, participants from Armenia, Azerbaijan and Ukraine were not able to attend the workshop due to visa problems. The participants of the workshop were mainly from molecular or seed testing laboratories either at agricultural universities, agricultural institutes, state or independent agencies or institutes for plant protection, variety and seed control as well as from plant inspection services. Many of the participants were trained in molecular biology; among them were head of laboratories for biotechnology, molecular biology or seed testing, some researchers and an inspector. The five participants from Slovenia were from seed testing

and molecular laboratories at AIS.

Dr. Kakoli Ghosh, Agriculture Officer for Capacity Building from the Seed and Plant Genetic Resources Service, FAO, Rome, attended the workshop as an observer.

## Workshop programme

The content of the workshop was divided into two parts, electrophoretic methods for varietal verification and PCR based methods for GMO detection; both were made up of lectures and practical work. The first part was led by Prof. Dr. Norbert Leist, Chair of ISTA GMO Task Force. At the beginning of his lectures he stressed the importance of assessing the quality of seeds before they are sown in order to prevent one of the greatest risks in agriculture, sowing the seed that does not have the capacity to produce an abundant crop of the required cultivar. Afterwards he introduced different methods used for the verification of species, cultivars and hybrids, each of them having a specific field of application. The principles and the applications of PAGE and SDS-PAGE electrophoresis, electrophoresis of isozymes and Isoelectric Focusing in Ultrathin Layer - IEF (UTLIEF) were presented in detail.

The practical part of work was led by Mr. Rainer Knoblauch, Vice-chair of the ISTA Variety Committee. With his feeling for explaining every little detail he led us through the whole process of IEF of seed storage proteins of *Zea mays*, *Triticum aestivum*, *Cucumis sativa* and *Oryza sativa*. Similar to all the other lecturers he offered the participants his help after the workshop via e-mail, if some questions arose when applying the knowledge in our own laboratories.

The increasing number of plant varieties that have been obtained through genetic manipulation grown worldwide raised the need for GMO detection. Different methods for detec-

tion of GMO exist, the PCR based methods have the largest field of application and are therefore the most widely used. Their principles and use were presented by Dr. Enrico Noli, member of ISTA GMO Task Force. Ms. Rossella Chiodini led the practical work from DNA extraction and quantification to screening and specific detection of genetically modified maize and soybean. Quantification was performed on Real Time PCR machine ABI 7000, which was provided especially for the workshop from the company Omega from Slovenia. As an alternative to Real Time PCR quantification subsampling quantification was presented as well.

## GMO testing in Eastern European countries

After the workshop I was asked by ISTA Secretariat to write an overview of GMO testing in Eastern European countries. In order to complete and broaden the information received during the workshop, I wrote a letter to all the participants with questions about the general situation in their own countries regarding legislation, growing and testing of GMOs. I did not receive answers from all the participants, some participants that I received the answers from were not informed in detail about the situation in their countries. The summary below is therefore only a brief reflection of the situation in this part of Europe.

The general situation regarding the GMOs in Eastern European countries can be divided into two parts, the situation in the countries that have recently joined the European Union (Estonia, Hungary, Latvia, Poland, Slovakia) and the situation in the countries that are not members of EU (Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Romania, Macedonia, Serbia & Montenegro and





Turkey). In the newly accessed EU countries the legislation concerning GMOs before accessing was already in place and it was in accordance with EU legislation, if not, they adopted EU legislation after accessing. In most countries there is no commercial cultivation of GM plants, in some countries there are field trials with certain GMOs (e.g. plum trees and cucumbers in Poland). In each country they have at least one laboratory performing GMO testing and at least one laboratory being member of the European Network of GMO laboratories (ENGL) (Latvia - one, Estonia, Hungary - two, Slovakia - three, Poland - five). Generally, the laboratories that test GMO food and feed are more advanced and better equipped than laboratories for testing seeds, but also these laboratories are equipped at least with the conventional PCR machines. Some laboratories are still in the phase of introducing the testing of GM seeds (Estonia, inspection service in Poland).

The situation is much different in countries that are not EU members. Many of them still do not have a regulatory framework concerning GMOs or it is not complete (e.g. Croatia, Turkey). In Macedonia, development of National Frames of Biosafety is introduced through an UNEP/GEF project. In Romania they have the law on the regime of testing, using and trading of GMO and of products resulting thereof. In the Official Directory of Varieties from Romania 14 varieties of GMO soybeans are registered. These varieties are allowed to circulate inside Romania only if they have written on the label and on their official quality document that they are GMO. At present there is no laboratory for GMO detection. The Central Laboratory for Quality of Seeds and Planting Material wants to build the first laboratory for GMO detection, but they have no funds for the moment. In Serbia and Montenegro there are already laboratories equipped for performing GMO analysis whereas in Bosnia and Herzegovina they are only establishing one. In Macedonia they are introducing GMO testing in the laboratory at the Faculty of Agricultural Sciences and Food. In Croatia no laboratory is authorized for GMO detection, for the moment GMO testing is performed in the Croatian public health laboratories. The laboratory at the Agricultural Institute in Osijek is preparing for GMO testing for their needs. In Turkey there is no permission to use GMOs, but illegally the GMOs are produced. There is no regulation concerning labelling. There is only one laboratory for GMO testing and it is accredited, another one is preparing for testing the seeds. They have the necessary equipment (PCR, electrophoresis) and will start introducing the methods.

I received the answer also from a colleague from Azerbaijan. He wrote that there is no cultivation of GMOs in Azerbaijan. Only one laboratory could perform GMO analysis in the future, but their problem is absence of modern equipment, although they have some people, that were well trained outside the country.

#### GMO testing in Slovenia

Slovenia is one of the newly accessed EU member states. Slovenian legislation related to GMOs implements or enforces the EU legislation and the Cartagena Protocol on Biosafety. Since 2002 we have the Management of Genetically Modified Organisms' Act, which provides a horizontal type of legislation on the use of GMOs and their products, and in this part, links up with the existing legislation in the areas of agriculture and health care. The Act regulates the contained use of GMOs, the deliberate release of GMOs into the environment, and placing on the market, importing and exporting GMOs or products containing GMOs or consisting of them or their combinations. There is no commercial growing of GMOs neither are there any field trials with GMOs in Slovenia. Four laboratories perform GMO testing, laboratory at the National Institute of Biology (NIB) (food, feed, seed), laboratory at the Agricultural Institute of Slovenia (seed), laboratory at the National Veterinary Institute (feed) and laboratory at the Institute of Public Health (food). The laboratory at NIB is accredited according to ISO 17025 standard for qualitative and quantitative analysis of GMOs. The laboratory at the Agricultural Institute of Slovenia followed the guidelines of ISTA and started with the introduction of methods for GMO testing in seeds in order to complete the chain in seed testing, which is performed in our ISTA accredited seed testing laboratory. We introduced the methods for qualitative detection, quantification is performed using the subsampling schemes or with renting the Real Time machine at a commercial company. The recently received grant will give us opportunity to purchase Real Time PCR machine at the beginning of 2005. At the moment we still do not perform regular testing, the laboratory at NIB has competitive advantage because of accreditation. The laboratory at NIB and our laboratory are both members of ENGL.

#### Conclusions

Hosting the workshop was a very good experience for

our laboratory. Having had excellent lecturers we gained a lot of new knowledge in the field of electrophoretic methods for varietal verification and identification; in the field of GMO testing many questions that were raised in our previous work were answered. From the organizational point of view it was a good experience on how to handle a larger group of people and how to organize the work in the laboratory. At the opening of the workshop responsible persons from Slovenian ministries were present as well, so hosting a workshop like this was also a good P.R. for the laboratory.

It was also a great opportunity for us to meet many people from different countries and different working conditions but sharing the common goal to perform seed testing as professionally as possible. Participants also provided an overview of the general situation on seed testing in their own countries. Feedback from the participants was more than positive, they were all satisfied with the workshop and they said the knowledge obtained would help them in their future work.

#### Acknowledgements

I would like to thank: ISTA and especially Dr. Bettina Kahlert, for giving us the opportunity to host the workshop and for all the correspondent support before and during the workshop. FAO for sponsoring the workshop. Prof. Dr. Norbert Leist, Dr. Enrico Noli, Mr. Rainer Knoblauch and Ms. Rossella Chiodini for transferring a whole lot of theoretical and practical knowledge not only to the participants but also to our group from the institute that participated at the workshop. Dr. Kakoli Ghosh for all her contribution. We discussed with her a lot during the workshop and a lot of ideas also for future cooperation were proposed by her. Company Omega from Ljubljana, Slovenia to make it possible that we got the Real Time PCR machine for the workshop. And last but not least, I would like to thank all the participants for their keen enthusiasm in attending the lectures and practical work as well as for their friendship gained in the time that we spent together. ■



# AVRDC/ARC/APSA/ISTA Seed Testing Workshop

Kasetsart University, Thailand  
August 23 - 27, 2004

By Anny van Pijlen, ISTA Technical Auditor

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The workshop held from 23 - 27 August at Kamphaeng Saen Campus in Nakhon Pathom, Thailand was attended by 21 participants from 7 different Asian countries namely: India, Japan, Indonesia, Vietnam, Bangladesh, Hongkong and Thailand.

At the opening session the director of APSA Dr. Sindhu was present and in his speech he emphasized the importance of seed testing workshops in the Asian region. The introduction speech was held by Dr. Suzuki, director of AVRDC. Training consultant Mr. Altroveros from AVRDC introduced the participants and explained the programme for the week to come and naturally some words were expected from my side.

The course was initiated and supported financially by APSA (Asia & Pacific Seed Association) and AVRDC/ARC (Asian Vegetable Research and Development Centre; Asian Research Centre). The coordination of the workshop was in the capable hands of Dr. Sutevee Sukprakarn and her colleagues.

Resource persons involved were Dr. Sutevee Sukprakarn, Dr. Sunanta and Mr. Altroveros from Kasetsart University, Thailand and Anny van Pijlen, The Netherlands, representative from ISTA. Lectures were prepared at NAK (General Dutch Inspection Service), samples for purity analyses and germination testing were prepared at the Kasetsart University in Thailand.

The participants were mainly staff of commercial seed companies. The training was a mix of lectures and practical classes. The documentation and the lecture about ISTA accreditation turned out to be of great interest for the participants.

Topics covered during the workshop were ISTA Accreditation, Seed Sampling, Purity analyses and Germination testing. Species were chosen from the Asian continent and were therefore relevant for the participants. (Tropical) species covered were: *Vigna unguiculata*, *Coriandrum sativum*,

*Lycopersicon esculentum*, *Glycine max*, *Oryza sativa*, *Spinacia oleracea*, *Solanum melongena*, *Zea mays*, *Ocimum basilicum*, *Lactuca sativa*, *Amaranthus*, *Celosia*, *Zinnia*, *Cosmos*, *Gomphrena*, *Lactuca sativa*, *Beta vulgaris*, *Allium cepa*, *Hibiscus esculentus*, *Cucumis sativus*, *Citrullus lanatus*, *Luffa* and *Brassica chinensis*.

On groups of species theoretical classes were held. On all species practical classes were held on purity and germination.

The workshop focussed on using the PSD (Pure Seed Definitions) in purity analyses and judging germination tests according to the recently issued ISTA Handbook for seedling evaluation.

The fact that the participants took part in the discussions in a lively way during the lectures and the practical classes was an indication that the workshop was successful.

The workshop dinner was held near the hotel premises and offered the participants to meet in an informal way. Some participants turned out to be good as well as in singing as in seed testing. There were even excellent magicians among the participants. And the participants will never forget the humorous contribution of Bangladesh. Thai participants showed how a traditional Thai dance was performed and of course the dance was brought into practice by all participants. Mr. Altroveros composed with the help of some students a nice slide show from pictures taken during the workshop.

The workshop started with noting down the personal expectations of the participants in relation to seed testing. It was splendid to come to the conclusion, at the end of the workshop, that

more than 90% of the expectations were obtained.

The participants express themselves that they have good confidence in carrying out the work with more enthusiasm in the future now that they have more profundity in relation to seed quality testing. The participants stressed that they have more tools now for passing on knowledge to other seed analysts. After increasing the knowledge during the workshop, participants were certain that they are able to implement the gathered knowledge in their daily work.

At the closing session the participants received a certificate of attendance, a CD with the lectures held during the workshop and an immense amount of pictures taken during classes. Summarising the workshop in one catchword: **successful**. ■



# ISTA Purity Workshop

## Budapest, Hungary

### May 11 - 12, 2004

By **Maria Rosaria Mannino**, ISTA Purity Committee Chair, GEVES, France, e-mail: maria-rosaria.mannino@geves.fr,  
**Zita Ripka**, ISTA Purity Committee Member and National Institute for Agricultural Quality Control, Hungary, e-mail: RipkaZ@ommi.hu,  
**Ken Allison**, ISTA Purity Committee Member Central Seed Laboratory, Canada, e-mail: kallison@inspection.gc.ca



**Figure 1. The participants of the ISTA Purity Workshop in Budapest.**

This year the ISTA Purity Committee organized a workshop which has been held at the National Institute for Agricultural Quality Control in Budapest. The workshop was a part of pre-congress ISTA activities. 33 participants were present coming from 22 different countries (Figure 1).

After the greeting of Dr. Katalin Ertsey, from the part of the National Institute for Agricultural Quality Control, Prof. Dr. Norbert Leist, ISTA President at the time, introduced the workshop. Dr. Bettina Kahlert, ISTA Secretariat, was present during the two days.

The following topics were discussed:

- 1. The Purity Committee: work and organisation**  
- Ken Allison
- 2. Discussion of ISTA Rules Chapter 3 and Annexe**  
- Maria Rosaria Mannino
- 3. The Universal List of Species**  
- Ken Allison
- 4. The use of Pure Seed Definitions**  
- Maria Rosaria Mannino
- 5. Seed scanner for determination of other seeds by number automatically**  
- Axel Goeritz
- 6. Seed mixtures**  
- Ken Allison
- 7. Difficulties of seed identification of *Medicago* spp.**  
- Susanne Andersen
- 8. Difficulties of seed identification of *Cucurbitaceae***  
- Zita Ripka
- 9. Difficulties of seed identification of *Bromus* spp.**  
- Maria Rosaria Mannino
- 10. Difficulties of seed identification of *Triticum* spp.**  
- Rita Zecchinelli
- 11. Blowing of *Poaceae* seeds**  
- Jette Nydam

In this article we relate only a part of topics discussed. A complete workshop report is in progress of writing.

**The Purity Committee: work and organisation (Ken Allison)**

Our meeting started with a presentation of the Purity Committee by Ken Allison, Chair of the Committee at that time. He presented the composition of the committee (10 members) and the organisation in 8 working groups: Pure Seed Definitions, Tropical and Subtropical Species, Proficiency Test Liaison, Identification of Seeds, Blowing, ISTA Website liaison, Workshop Development, Seed Mixtures. The work done and future aims were also discussed. The proposed list of 130 species that all seed testing stations would be expected to retrieve and identify and of a procedure to test seed mixtures are among the major accomplishments of the Committee. For the future, the Committee will be involved on the tasks of redoing the Pure Seed Definitions handbook, finalizing the "Universal List of Crops and Weeds", studying the relation between the size of the calibration sample and that of the working sample, formalising the basic outline to be followed for all purity workshops, and others discussed during the congress.

**Discussion of ISTA Rules Chapter 3 and Annexe (Maria Rosaria Mannino)**

During this session we read Chapter 3 and its Annexe, which allowed us to discuss, paragraph by paragraph, the part of ISTA Rules concerning purity, and to bring out all difficulties of interpretation in applications.

Main points gave rise to a detailed discussion or correcting suggestions. The participants underlined the need for clarification of *Cuscuta* seeds identification as "inert matter"

or "other seeds" in relation to their physical characteristics. The Rules are not currently clear on this point (Figure 2). We also discussed the need to specify in the ISTA Rules the tolerances for working sample weights and the interest in classifying *Hordeum* as "non chaffy" instead of "chaffy". A concern was raised about the difficulty in counting seeds in capsules or schizocarps (e.g. *Papaver* and *Malva*).



**Figure 2. *Cuscuta* seeds with different characteristics of structure and colour.**

**The Universal List of Species (Ken Allison)**

Ken Allison presented the Universal List of 130 species (crops and weeds), drawn up by the Committee and currently under discussion within ISTA. He explained how this list was developed: on the basis of the answers to a questionnaire sent to ISTA laboratories, a selection was made taking in account the number of laboratories and their rating of each species. Different applications will be possible: the ISTA Proficiency Test Committee and test leaders could use this list for referees; audit teams could expect all accredited laboratories to have the listed species as a minimum requirement in their reference collections. On these points and other

possible uses, a decision has to be made by ISTA. The Purity Committee plans to finalise the list and to organise a proficiency test to familiarise laboratories with the species. Another important task will be to find sources of seeds for distribution to laboratories to add to their reference collections.

### The use of Pure Seed Definitions (Maria Rosaria Mannino)

We tackled this subject by reading the PSDs and discussing them. For some PSDs, we also did practical exercises (Figure 3). During this session we discussed PSD numbers 1 (*Cannabis*), 4 (*Cichorium*, Figure 4), 10 (*Pisum*, *Brassica*, Figure 5), 21 (*Onobrychis*), 23 (*Raphanus raphanistrum*, Figure 6), 25 (*Valerianella*), 28 (*Cynodon*), seeds with attached appendages (15, 38, 46, 47 and 62). PSD have been chosen by the workshop organisers on the basis of their importance (number of genera covered or difficulty of use) and also following suggestions of participants. In some cases, during the session it appeared the interest of a modification of PSD; e.g. *Cichorium*, can have attached bracts but these appendages are not mentioned on the current PSD 4.



Figure 3. Practical exercise during the session on PSD.



Figure 4. Bracts can be attached to the seed of *Cichorium* spp., but PSD 4 mentions only the pappus and the beak as appendages to be found on the seed.

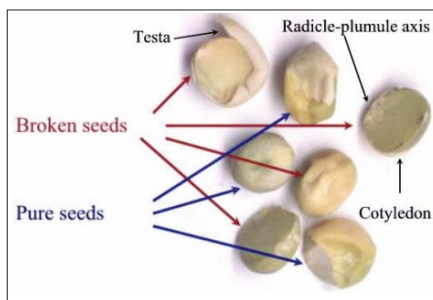


Figure 5. For seeds of the *Fabaceae* family, the presence of testa is relevant to evaluate seed purity. A separate cotyledon is regarded as inert matter irrespective of whether or not the radicle-plumule axis and/or more than half of the testa is attached.

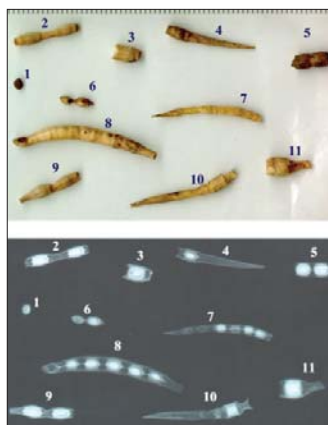


Figure 6. Siliqua of *Raphanus raphanistrum*: as we see it and under X-ray. The presence of seed is relevant to evaluate seed purity, but sometimes it is difficult to evaluate the presence of seed on the siliqua.

### Seed mixtures (Ken Allison)

Purity Committee members from several countries provided the protocols in use in their system for consideration. On this basis, some modifications of different chapters of the ISTA Rules have been proposed and illustrated by Ken Allison who was the leader of the ISTA working group on Mixtures. For the future, a handbook will be prepared for use of laboratories analysing mixtures and the sampling, in particular, will be studied.

### Difficulties of seed identification of *Medicago* spp. (Susanne Andersen), *Cucurbitaceae* (Zita Ripka), *Bromus* spp. (Maria Rosaria Mannino) and *Triticum* spp. (Rita Zecchinelli)

This part of the workshop included four lectures and practical exercises on difficulties of seed identifications. The corresponding PSD

and some relevant characteristics of seeds were presented with the aim of distinguishing different species. Documents and seed collections were distributed to the participants. We started with *Medicago* species (*M. lupulina*, *M. sativa*, *M. orbicularis*, *M. polymorpha*, *M. rigidula*) and other similar species (*Melilotus* spp., *Trifolium pratense*) presented by Susanne Andersen. During the practical exercises, two samples of *Medicago sativa* and *Medicago lupulina* were analysed. Zita Ripka presented the descriptions of 13 different species of *Cucurbitaceae* (Figure 7). *Cucumis sativus* and *Cucurbita pepo* were analysed by the participants.



Figure 7. The most frequently cultivated *Cucurbita* seeds.

The lecture on *Bromus* species, prepared by Maria Rosaria Mannino, showed the descriptions of 11 species of *Bromus* (Figure 8); purity analysis was done on samples of *Bromus catharticus* and *Bromus inermis*. Rita Zecchinelli spoke about *Triticum* species. The most important characteristics to distinguish between *T. aestivum* and *T. durum* were shown. We discussed also the difficulties related to varietal characteristics that can make the distinction difficult (Figure 9).



Figure 8. The retrieval of *B. sitchensis* (left side) on samples of *B. catharticus* (right side) can present some difficulties. The seeds of these species can be the same size, but they differ in shape, nerves of the lemma, colour of the caryopsis, size of rachilla.



Figure 9. *Triticum durum* in the middle, two different varieties of *Triticum aestivum* on the right and on the left. Some varieties of *T. aestivum* have vitreous endosperm.

**Blowing of *Poaceae* seeds (Jette Nydam)**

During this session, several points have been explained and discussed: blower characteristics, setting, calibration and re-calibration, special cases and problems. Some exercises of calculation were done on the determination of the uniform blowing point on the basis of the number of misplaced florets.

During the meeting, participants visited the purity laboratory of the National Institute for Agricultural Quality Control (Figure 10).



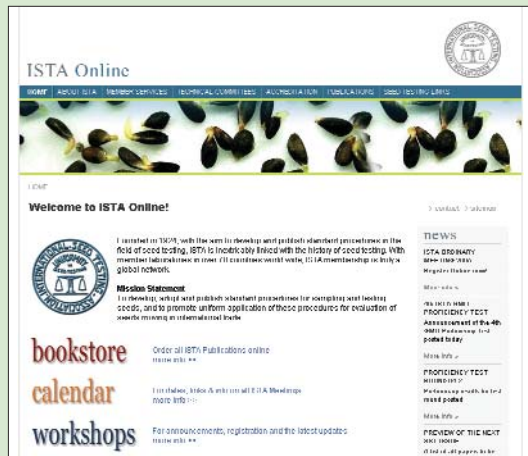
Figure 10. Zita Ripka with participants during the visit of the purity laboratory of the National Institute for Agricultural Quality Control, Budapest.

The workshop was much appreciated by the participants: many points of daily analyst work were discussed and different experiences compared.

During the Congress, the Purity Committee planned to organise three Purity Workshops: in 2005 (Asia), 2006 (Europe) and 2007 (America). Contacts are in progress to find the countries and establish the dates. ■



# ISTA launches its new website in May 2004



ISTA officially launched its new website in May 2004, just in time for the 27<sup>th</sup> ISTA Congress. Although the address remains the same, the website changed dramatically. Whether you are searching for information on ISTA Membership, quality control and accreditation, or information on the work of the ISTA Technical Committees, it is all readily available on the internet.

All publication can be ordered online, and many have been using the online registration option for the various workshops.

With the ISTA Ordinary Meeting 2005 on the horizon, we are sure that all members and associated persons will be interested to hear that all meeting documents will be available for download on the website, and registration for the Ordinary Meeting was available since October 1st, 2004.

A new aspect of the accreditation pages includes the latest up to date information on Proficiency Tests. As the information becomes available, it is immediately posted to the website. If you are looking for the introduction and instructions, or the results from the latest Test Round, or would like to download the report sheet, it is all available at the click of a mouse.

Any comments or suggestions regarding the website will be greatly appreciated. Just direct them to the webmaster.

Keep up to date, and informed, visit [www.seedtest.org](http://www.seedtest.org)

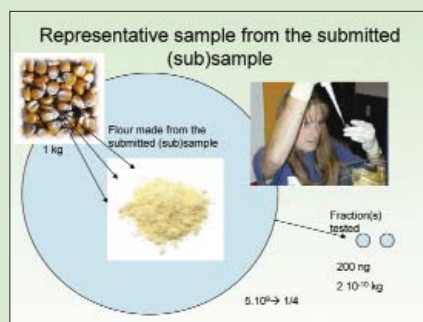
visit ISTA Online at  
**[www.seedtest.org](http://www.seedtest.org)**

# ISTA Workshop on Statistical Aspects of GMO Detection

St Louis, Missouri, United States

November 17 - 19, 2004

Sylvain Grégoire, Kirk Remund and Jean-Louis Laffont, ISTA GMO Statistics Task Force Members



**Organiser** - ISTA STA-GMO Task Force and ISTA GMO Task Force Proficiency Test Group

**Location** - Monsanto, St Louis, Missouri, United States

**Onsite Hosts** - Doris Dixon and Kirk Remund

ISTA is organizing a workshop with the aim to help laboratories to address the test planning, and the use of results, on GM detection in seeds. The objective of the workshop is to give and exchange information on matters that have been mentioned by laboratories which already participated to the ISTA Proficiency Tests, or intend to join.

Lectures will be accompanied by practical use of software which is available on the ISTA website, such as Seedcalc for instance.

Not only ISTA Proficiency Tests will be addressed, but GM tests on seeds in general.

Other ISTA Workshops deal with the technical aspects of detection by laboratories; laboratory technique will not be demonstrated in this workshop.

The workshop will run from 8.00am, November 17<sup>th</sup> to 5.00pm, November 19<sup>th</sup>.

**Workshop Content**

- Obtain an estimate of % of GM in a sample
- Introduction on uncertainty of measurement using ISO 17025
- Robustness of testing plans (tolerance to

- false positive and/or false negative rates)
- Determination of appropriate testing plan(s) to thresholds or quality levels
- Check of purity of reference material
- DG SANCO testing plan for GM seeds (example of test in two steps)
- Repeatability (measure variability in a lab) and reproducibility (measure variability in a set of labs) for quantitative results using ISO standard 5725 (quoted in ISO 17025)
- Distributions usually found in living material and used in computations (Binomial, Normal, ...)
- Data checking (outliers or "suspected error" points)
- Limit of detection, limit of quantification
- Example of computations from raw results obtained in a test
- Principle of statistical test and associated risks (alpha, beta) test used as an example

**Practical Work**

All participants will practice computer use software on simple examples. Several computers will be provided for use during the workshop but participants are allowed to bring and use their own computers if they desire.

**Presenters of the Workshop**

The workshop will be presented by Sylvain Gregoire (France, GEVES), Kirk Remund (USA, Monsanto) and Jean-Louis Laffont (France, Pioneer) who are the core of the ISTA STA/GMO TF (sub-group of the ISTA Statistics Committee dedicated to help the ISTA GMO Task Force, in particular the Proficiency Test Working Group).

**Location**

The workshop will take place at the Monsanto Company in St. Louis, Missouri, US.

**Registration**

The number of participants is limited to a maximum of 20. *Unfortunately the workshop already has the maximum number of participants.*

# ISTA Seed Quality Assessment Training organised by APSA

Hanoi, Vietnam

November 22 - 26, 2004

APSA, ISTA, and the Danish International Development Agency (DANIDA) invite you to attend the Training Course on ISTA Seed Quality Assessment.

The course will be held on 22-26 November 2004 in Hanoi, Vietnam. The training course is intended for people working on seed production and/or seed quality testing in government office or private seed companies from the Asian Pacific region. It is designed to provide participants with the knowledge and skills to effectively assess seed quality aspects, understand better seed quality assessment system, and improve the present system to meet international standards.

The training will be lectured Prof. Dr. Norbert Leist and Ms. Andrea Jonitz (LUFA Augustenberg, Germany) from ISTA.

The training course will have three major subjects:

1. Seed sampling
2. Seed purity analysis
3. Seed germination test.

The course fee will be covered by APSA but participants should pay for their airfare to and from Hanoi and for their board and lodging. ■

For further details, please contact the APSA Secretariat:  
 P.O. Box 1030  
 Kasetsart Post Office  
 Bangkok 10903, Thailand  
 Tel: +66-2-940-5464  
 Fax: +66-2-940-5467  
 E-mail: [apsa@apsaseed.com](mailto:apsa@apsaseed.com)

# 5<sup>th</sup> ISTA SHC Seed Health Symposium

Angers, France, May 10 - 13, 2005

Joël Léchappé, ISTA Germination Committee Chair



The Organising Committee is proud to invite you to the 5<sup>th</sup> ISTA SHC Seed Health Symposium from 10-13 May 2005 in Angers. The work programme will focus on the technical and scientific aspects of seed health testing and seed-borne diseases.

We will do our best to ensure a pleasant stay. Angers is a unique setting in terms of history and modern technologies. Located in the Loire Valley, it will provide an ideal environment for scientific exchanges and conviviality.

It will be our pleasure to welcome you and your family.

Joël Léchappé  
President of the Organisation Committee

## Preliminary Programme

### - Tuesday, 10 May

Afternoon - Opening plenary session: discussion of the new SHC structure and working groups

Initial meeting of working groups

### - Wednesday, 11 May

Morning - Session I and Keynote presentation

Afternoon - Session II

Symposium Dinner

### - Thursday, 12 May

Morning - Session III Regional Seed Health Issues

Afternoon - Plenary session

### - Friday 13, May

Morning - Steering Committee and meetings of Working groups

## Organisation

### - Hosting Institute

GEVES (Groupe d'Étude et de contrôle des Variétés et des Semences)

### - Organising Committee

GEVES, INRA, Angers University, Plant Health Service/National Laboratory for Plant Health

### - Registration

GEVES - SNES

Rue Georges Morel - BP 90024  
49071 Beaucouzé Cedex - France  
Tel. 33 (0)2 41 22 58 03  
Fax 33 (0)2 41 22 58 01

Registration forms may also be downloaded from the ISTA Website at: [www.seedtest.org](http://www.seedtest.org)

## General Information

### - Date and venue

The 5<sup>th</sup> Seed Health Symposium will take place from May 10 to May 13, 2005 at the Angers Congress Centre which is in the very heart of the city, in walking distance from historical sites and from the railway station.

### - Languages

The working language of the Symposium will be English. There will be no translation.

### - Invitations

The Organising Committee will be happy to send a personal invitation to any participant upon request to the registration secretariat. Such invitations may help participants to make their travel and visa arrangements, but are not a commitment on the part of the Organising Committee to provide financial support.

### Papers

The organisers invited papers or posters based, either on research or practical experience from the list of topics given.

## List of topics for the ISTA Seed Health Symposium:

- Innovations in seed health testing
- New diseases and emerging seed-borne pathogens

- Methods of standardization and evaluation of comparative tests
- Quality assurance in seed health testing
- Seed contamination from infected plants
- Chemical and physical seed treatment
- Seed health and the international movement of seed
- Other topics (please precise)

## Travel information

### - Access to Paris

By air:

International airlines offer connections to the Paris Airports (Roissy Charles de Gaulle and Orly airports).

Both offer public shuttle to the city centre (90 mn).

By rail:

Direct intercity rail links with all major French cities and all European destinations.

### - Access to Angers

- From Paris

By train (note that an advance seat reservation is necessary)

Direct TGV (high speed train) from Roissy Charles De Gaulle Airport (2h30)

15 daily direct TGV leaving from Paris at Montparnasse Station (90 min).

By car Paris - Angers: 300 km by motorway A11 (2h30).

- From Nantes International Airport (100 km from Angers)

By train TGV from Nantes railway Station to Angers (30 min).

By car Motorway A11 (60 min).

Possibility to rent a car or to hire a taxi from Paris, Nantes.

## Accommodation

Prices range from approximately 40 to 97 Euros for a single room and from 42 to 110 Euros for a double room, breakfast extra. ■

# 7<sup>th</sup> Seminar on Statistics in Seed Testing

University of Hohenheim, Stuttgart, Germany

August 29 - September 2, 2005

Michael Kruse, ISTA Statistics Committee Member



**Location**

University of Hohenheim  
 Institute for Plant Breeding, Seed Science  
 and Population Genetics  
 70593 Stuttgart  
 Germany

**Participants**

25 at maximum per part  
 Participation fee not yet decided

Are you interested in participation?

Please fill out the preliminary  
 registration form below and send it  
 to Michael Kruse,  
 University of Hohenheim 350D,  
 D-70593 Stuttgart,  
 Germany

**Preliminary Programme**

*Monday and Tuesday*

Workshop on practical applications of  
 statistics in seed testing e.g. on  
 - tolerances  
 - proficiency tests  
 - seed calc ....

*Wednesday*

Presentation and discussion of STA  
 work  
 Excursion

*Thursday and Friday*

Scientific Seminar  
 - new statistical approaches for seed  
 testing  
 - statistics of GMO testing  
 - statistics for scientific research ...



Yes, I am interested to participate in the 7<sup>th</sup> ISTA Seminar on Statistics 2005 and would like to receive further information

Last Name ..... Address .....  
 .....  
 .....

First Name ..... Country .....

Institution / Company ..... Phone .....

..... Fax .....

..... E-mail .....

.....



# News from the ISTA Proficiency Test Committee

By **Günter Müller**, ISTA Proficiency Test Committee Chair and **Martina Rösch**, Head of ISTA Accreditation, Thüringer Landesanstalt für Landwirtschaft TLL, Germany, e-mail: g.mueller@jena.tll.de

## Proficiency Test Programme Plan 2004-2007

The ISTA Proficiency Test Committee plans to organize 11 proficiency tests rounds in the triennial period between the ISTA Congresses. One of them will be a proficiency test with flower seed. The test rounds with *Secale cereale* and *Medicago sativa* are arranged in cooperation with the Moisture Test Committee. The participants shall determine the moisture content of these two species.

With *Cynodon dactylon* and *Panicum maximum* we will have two species from the tropical region in our test programme. It is intended to perform a biochemical test for viability (TZ) on *Panicum maximum* and *Medicago sativa*. The Vigour Committee plans to include a conductivity test for peas in conjunction with the test round 05-3.

## Schedule

Round	Distribute	Species	Tests*
04-3	October 2004	<i>Phleum pratense</i>	P, G, OSD, OIC
05-1	February 2005	<i>Cynodon dactylon</i> <i>Zinnia</i>	P, G, OSD G
05-2	June 2005	<i>Secale cereale</i>	P, G, OSD, MOI, OIC
05-3	October 2005	<i>Capsicum annuum</i> <i>Pisum sativum</i>	G VIG
06-1	February 2006	<i>Sorghum bicolor</i>	P, G, OSD, OIC
06-2	June 2006	<i>Beta vulgaris</i>	P, G, OSD
06-3	October 2006	<i>Phaseolus vulgaris</i>	G
07-1	February 2007	<i>Panicum maximum</i>	P, G, OSD, OIC, TZ
07-2	June 2007	<i>Medicago sativa</i>	P, G, OSD, MOI, TZ
07-3	October 2007	<i>Raphanus sativus</i>	P, G, OSD

\*P = Purity, G = Germination, OSD = Other Seed Determination, OIC = Orange International Certificate, TZ = Tetrazolium, MOI = Moisture, VIG = Vigour

## Rating System

The first round evaluated according to the new rating system was the proficiency test with *Pisum sativum* in 2002. In the meantime the final results of two test rounds are available. The PT Committee evaluated the first two test rounds in order to determine suitability and appropriateness of the rating system.

A high number of in-round ratings were below minimum performance (BMP), in part due to the fact that all three germination components (normal, abnormal, non-germinated) were included in the calculation and thus laboratories were rated for deviations that are not mutually independent. Therefore it was decided by the committee to only take the Z-scores of the normal seedlings into consideration when calculating the laboratory's in-round score. Z-Scores for the components abnormal and non-germinated seed will be reported in order to enable the participating laboratory to evaluate the test results. In addition, the PT Committee concluded that the second component of rating the laboratory's performance, i.e. the number of Z-Scores outside +/- 2.0 shall be omitted as it complicates the system and does not add any value. The aim shall be a fair and transparent rating system that enables to identify poor performance.

Thus, a revision of the in-round rating system was developed which shall be as follows:

### Z-scores for germination testing (normal seedlings only)

Score	Sum of absolute Z-scores
A	<3.5
B	between 3.5 and 5.3
C	between 5.3 and 7.0
BMP	>7.0

The two test rounds that have already been reported to the participants will be re-evaluated according to the changes described and reported to the participants.

Regarding other seed determination (OSD) it was decided to consider retrieval and identification of seed separately. The PT Committee is working on a new rating system which takes into account the laboratory's ability to retrieve and identify other seeds added. The document 'The ISTA Proficiency Test Programme' shall be revised in due course in order to reflect latest changes. ■

## News from the Secretariat

The ISTA homepage is a invaluable tool for disseminating relevant information to members and interested parties. The Accreditation Department is working on improving and expanding availability of useful information regarding the Proficiency Test Programme on the ISTA Website. With test round 04-2, for the first time, the proficiency test instruction letter and report form were provided on the internet for download. Participants are now able to use the electronic copies to fill in test results and may submit them by e-mail.

Some participants expressed their wish for a more immediate feedback on the test rounds results in order to commence investigation on potential sources of error. Thus, preliminary test results of test round 04-1 were sent to the participants in July 2004. The laboratories were provided with the mean results of the heterogeneity tests conducted by the test leader prior to the sample shipment. These results shall allow

for a preliminary evaluation of test results for orientation.



From now on, such a preliminary test report shall be provided on the ISTA Website for download once the deadline for submitting test results has expired. Other relevant information shall be provided as the need arises.

The ISTA Secretariat would like to encourage the use of the ISTA Website to make it an effective and efficient tool.

# Report from the ISTA Auditor's Meeting

Budapest, Hungary, May 16, 2004

By Gerhard Schuon, ISTA Accreditation Department



Since 2001 ISTA Auditors regularly meet at the occasion of an (Extra-) Ordinary Meeting. It was thus the fourth time technical and system auditors gathered to exchange views and experiences and to look back on the past year's assessment activities.

Beside the social and technical aspects and its salient element of training, this annual meeting was an excellent opportunity to contribute to the audit programme's performance in view of consistency and uniformity. Auditors' meetings are not intended to develop requirements for laboratories in order to be accredited but to strive for a maximum of harmonisation between the different assessment teams in evaluating audit observations. In addition, feedback from accredited laboratories is taken into account, and lessons learnt from previous assessments are presented to colleagues. Where necessary, discussions are summarised and presented in this format or they will lead to a specific document.

The agenda covered issues like the administration of audit activities, auditors' training and specific technical or organisational requirements from the ISTA Accreditation Standard that had given rise to questions during typical conformity assessments. Michael Muschick presented a summary on accreditation related discussions and decisions from the Executive Committee Meeting in New Zealand, earlier this year.

Following a decision of a previous auditors' meeting the different sampler monitoring approaches encountered during on-site assessments of the more recent past were looked at. The diversity of laboratories is

reflected in a variety of monitoring programmes. They all aim at correct execution of sampling and at providing confidence that the samples do truly represent the respective seed lots. The elements that are used in different combination and with varying emphasis comprise training, auditing and check sampling. Whereas annual auditing of sampling is obviously a requirement of the standard, check sampling is clearly an option that may be chosen or not. The issue was clarified on the understanding that:

- all individuals performing sampling for the issuance of ISTA International Seed Analysis Certificates have to be included in a laboratory's monitoring programme

- internal audits of sampling have to be conducted at least annually; the standard does not specify to what extent each sampler has to be involved in this

- check sampling, i.e. two or more separate sampling operations on the same lot in order to compare the samples' characteristics, can be a suitable measure to evaluate sampler's performance; intensity and frequency are a function of the supervision done by other means

- individual supervision and monitoring activities may be combined to increase efficiency

In the past, ISTA Accredited Laboratories had been asked to have a record system that ensures that analysis data can be related to the relevant sample without disclosing the customers identity to the personnel performing the tests. Since more and more laboratories do have a very limited number of customers for ISTA Seed Analysis

Certificates, features from the sample, e.g. species or variety, specific analysis or reporting requirements, may often reveal a customer's identity. In these cases, making work cards anonymous does not provide additional benefits while causing additional work. The auditors agreed that the Accreditation Standard requires a laboratory to demonstrate how it ensures integrity of its analytical work. However, anonymous laboratory work cards may contribute in achieving this, but they are not a requirement in their own right.

During the discussions many different views were set forth. In the spirit of team effort and responsibility towards the objectives of ISTA, the auditors could reach agreement in all points addressed. One outcome of the meeting was a set of activities that will be followed-up in the coming months in order to improve and fine-tune the work in ISTA's conformity assessments.

Amongst others, the audit feedback form will be amended to enlarge its scope and give the laboratories an opportunity to comment directly on any aspect of the accreditation procedure, from its inception over the various interactions with ISTA staff involved to the overall Accreditation Programme.

Martina Roesch, as the chairperson, closed the meeting with an acknowledgement of the valuable contributions from the technical auditors and of their commitment towards ISTA demonstrated throughout the year. She extended her thanks to the laboratories and organisations facilitating the work of the Accreditation Department by their support. ■

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## Professor Dr. Murray Hill retires from ISTA after 40 years of service



Forty years of service on ISTA Technical Committees came to an end in Budapest 2004 when Professor Dr. Murray Hill from New Zealand retired from the Storage Committee. Professor Hill joined the then Seed Moisture and Storage Committee of

ISTA in 1964 under the chairmanship of Dr. Oren Justice (US) and also became a member of the Working Group on Storage of Seeds of Temperate Climates. When the Seed Moisture and Storage Committee separated into the Seed Moisture and Seed Storage Committees, he continued as a member of both, working closely with Dr. Don Grabe (US) on the Seed Moisture Committee and Professor Patricia Berjak (South Africa) on the Storage Committee. He retired from the Seed Moisture Committee in 1998.

Professor Hill is known internationally for his seed technology teaching, research and training programmes, both in New Zealand and in the Asia/Pacific region (Thailand, Philippines, China, Indonesia, Malaysia and Australia). He and his colleagues have a seed technology alumni of nearly 1500 people from 64 countries who have trained at short course, certificate, graduate or postgraduate level both in New Zealand and internationally. This expertise and experience was long ago recognised by ISTA, and Professor Hill has provided keynote addresses at the ISTA Congresses in Vienna (1980), Brisbane

(1986), Edinburgh (1989) and most recently Budapest (2004). Along with his wife Karen, Professor Hill represented ISTA and provided the teaching/course content for an ISTA/APSA/AVRDC Seed Testing Workshop in Thailand last year.

Over the years Professor Hill has been the Director of three New Zealand ISTA member laboratories (NZDL01 from 1968 - 1972; NZDL02 from 1994 - 1997 and NZDL04 since 1998), and is currently Professor of Seed and Crop Science and Director of the New Zealand Seed Technology Institute at Lincoln University. While he has retired from ISTA committee work, he still maintains a strong interest in ISTA, through his current involvement with NZDL04 and also as Managing Director of the company that runs AUDL02.

Forty years of experience is a lot to lose from a Technical Committee, but active participation for this time in ISTA's work is to be applauded. ISTA sincerely thanks Professor Hill for that participation, and also his involvement in other ISTA activities over the years. His contributions will be missed. ■

## Dr. Abdul Rauf Bhutta, Deputy Director, FSC&RD offered life time award of Private Schools Network, Islamabad

Dr. Abdul Rauf Bhutta is a renowned Agriculture Researcher and Educationalist. He is working as Dy. Director, Federal Seed Certification & Registration Department, Ministry of Food, Agriculture and Livestock, Islamabad. He that have published 15 books and more than 78 research papers in National and International Scientific Journals. Apart from his scientific engagements, he is honorary working on promotion of education and organisation of private schools in rural areas of Islamabad for the last 20 years. Based on his contribution and dedication, Private Schools Network, Islamabad, embraced Dr. Bhutta with LIFE TIME AWARD. This award was presented to Dr. Bhutta by Senator Razina Alam Khan, Chairperson, Senate Standing Committee on Education, Government of Pakistan, Islamabad.



Dr. Abdul Rauf Bhutta, Deputy Director, FSC&RD, Islamabad Receiving **LIFE TIME AWARD OF PRIVATE SCHOOLS NETWORK**, from Senator Razina Alam Khan, Chairperson, Senate Standing Committee on Education, Governemnt of Pakistan. Prof. Abdul

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## MEMBERSHIP

### Request Form

#### International Seed Testing Association

- develops, adopts and publishes standard procedures for sampling and testing seeds
- promotes uniform application of these procedures for evaluation of seeds moving in international trade
- promotes research in all areas of seed science and technology
- Accredits Member Laboratories
- to participate in conferences and training courses
- has established & maintains liaison with other organisations having common or related interests in seed

#### ISTA Membership offers you

- ✓ free access to the 'International Rules for Seed Testing', an internationally standardised publication containing seed testing procedures and techniques, which is constantly revised and updated
- ✓ valuable information through all ISTA publications, including Seed Science Technology and Technical Handbooks, which are free for members
- ✓ involvement in seed testing methodology development
- ✓ ISTA proficiency testing, quality assurance standards and auditing services, which assist you in attaining the highest quality assurance levels in today's business environment
- ✓ the possibility of issuing ISTA international certificates
- ✓ easy access to leading seed experts worldwide

"ISTA, providing methods & services  
for the testing of seed moving  
in international trade..."

# ISTA



International Seed Testing Association  
Association Internationale d'Essais de Semences  
Internationale Vereinigung für Saatgutprüfung



## REQUEST FORM

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 fax +41 1 838 6001, E-mail [ista.office@ista.ch](mailto:ista.office@ista.ch) to receive a membership information package.



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

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Phone \_\_\_\_\_

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Nyon  
10 June 2004



# ISF Press Release

## ISF Congress in Berlin attracts participation from 58 countries

Representatives of the seed industry in 58 countries totaling 1284 delegates and accompanying persons participated in the 2004 annual ISF Congress in Berlin from 24-26 May 2004. The regional - European, East European, Asia-Pacific, South American and African - seed associations were also represented. Twenty-four exhibitors displayed products ranging software to sophisticated agricultural machinery.

Representatives from FAO, ISTA, OECD and UPOV attended the congress. ISF welcomed the decision taken at the ISTA congress that allowed all ISTA accredited private, public and company laboratories to issue orange certificates.

The congress got off to a good start with a keynote speech by Philip Freiherr von dem Bussche, President of the German Agricultural Society. In speaking of global responsibility and the role of plant breeding Mr. Von dem Bussche said, "The more intensively one considers global challenges, the more clearly one comes to the conclusion that there is hardly any aspect of sustainable development that appears soluble without modern farming and plant breeding".

Trading was brisk. Technical meetings were well attended and traditionally the first was the Breeders Committee where among others matters related to intellectual property and sustainable agriculture were discussed. Two position papers were debated and presented to the general assembly for adoption. The Vegetable and Ornamental Section meeting drew large numbers and a position paper on the definition of terms describing plant reaction to pests and pathogens was adopted. Guidelines for the handling of a dispute on essentially derived varieties of lettuce were also adopted.

The general assembly on 26 May 2004 adopted 4 position papers in all and amendments to the ISF trade and dispute settlement rules. They can be viewed on the ISF website.

Following the debates in the general assemblies of 2002 and 2003 on the complexity of the questions related to intellectual property protection of plant related inventions and access to plant genetic resources for plant breeding and varietal development, an international seminar on Protection of Intellectual Property and Access to Plant Genetic Resources took place on 27 - 28 May 2004. Specialists spoke of the evolution and intersection of the two topics. 175 persons from the public and private sectors in industrialised and developing countries participated. The proceedings will be published soon.

### For more information, contact

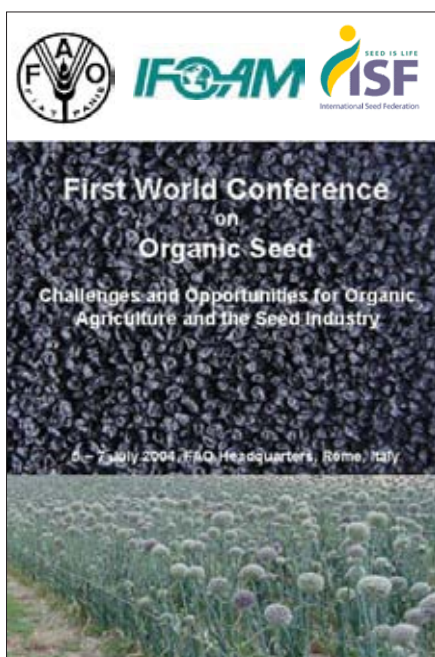
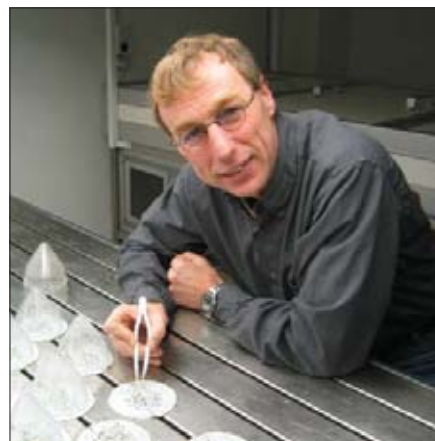
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# 'Challenges and Opportunities for Organic Agriculture and the Seed Industry'

A note on the First World Conference on Organic Seeds held at FAO Headquarters in Rome, Italy  
July 5 - 7, 2004

By Steven P. C. Groot,

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Last July we were guests at the FAO building in Rome, attending the First World conference on organic seeds. The conference was jointly organised by the International Federation of Organic Agricultural Movements (IFOAM), the International Seed Federation (ISF) and the Food and Agriculture Organisation of the United Nations (FAO). The meeting was very well organised and had attracted around 270 participants from 57 countries, showing the world-wide interest in organic agriculture and the importance of seeds. This diversity in participants was also presented in the mixture of farmers, seed company staff, small scale seed producers, policymakers and

scientists. The theme of the meeting was 'Challenges and Opportunities for organic Agriculture and the Seed Industry'. I was privileged to present ISTA on this meeting and talk on the issues of seed quality and the role of seed scientists in working on solutions.

The meeting discussed many technical, economical, social and political aspects of organic seed supply. In his opening speech Swedish organic farmer and IFOAM president Gunnar Rundgren stressed the challenges for the organic movement regarding seeds, the need for harmonisation in the organic sector, seed regulation in general and the importance of respecting farmers rights. Representatives from seed companies and public researchers demonstrated several examples of the efforts and challenges for organic seed production, aiming at the production of high quality organic seeds. A special session was devoted to the co-existence of genetically modified (GM) crops and the risks of GM contamination during organic seed production.

## Use of organic seeds?

"Should organic farmers use organic produced seeds as crop starting material?" was one of the main questions at the conference. Unintentionally, Mr. Rundgren induced confusion about the opinion of IFOAM whether organic produced seeds should be used and if certification for that organic production is required. Mr. Rundgren stated that the use of certified organic seeds should be a voluntary option, not a mandatory demand, "The use of certified organic seeds or not comes longer down on the list of my priorities, and also on

the priorities of the consumers buying my food". Questioned by several participants, he stated the next day that he preferred that organic seeds are used, whenever available. These confusing statements reflect the situation within the organic agricultural sector. Organic farmers worldwide prefer to use organic seeds, but they encounter several problems in that. Not always are organic produced seeds available, and if available they may not be certified as produced organic. The latter is frequently the case in developing countries where farmers use seeds from own production or from exchanges within the community. Moreover, for several crops it is still difficult to reach the same quality standards as for conventional seeds.

## Organic seed supply

In Europe, North America, Israel and New Zealand certified organic seeds become more and more available, but the higher costs may make it economically difficult to use them for certain crops. Ronald Peerenboom, chairman ISF Working Group Organic Seeds, showed how the seed companies have invested in organic seed production: "They have a vision, supported by the ideological and political will of the last decade that organic seeds might become an interesting 'niche' seed-business to be involved in. They learn from it and use the new experience also in breeding and production of conventional seeds. They understand the ambivalent attitude from the organic farmer, especially when the prices of organic produced seeds are high". Representatives from seed companies Hild, Enza and Bejo asked for clarity from the organic sector and if decided to go for the use





tion, the seed crop may be grown in a region where the crop is not grown widely and cultivation methods can aid in restricting spread of diseases. Genetic or variety aspects can influence seed production. Varieties that are chosen on good performance in organic

vegetable production do not always perform well during organic seed production. This can provide serious problems with some biennial crops or F1 hybrids.

Whereas the larger seed companies have well trained

staff for production of high quality seeds, it is a problem with the small scale seed productions and farm saved seeds in third world countries. Moreover, certification of seed quality hardly exists for these seeds. There were several strong questions towards ISTA for support on this aspect. The pamphlet that ISTA presented, on its role in organic seed production was highly appreciated. As I understand the pamphlet has also frequently been downloaded from the ISTA web site in the weeks after the conference.

of organic seeds to get clear deadlines for derogations. Seed companies need to plan their seed production two or three years ahead and large costs are involved. As Dick van Zeijden from Bejo stated, "The organic seed programmes will only have a future if the organic chain is closed by law and self regulation. Commitment of all parties is needed. If there will be no self regulation it will be difficult to maintain an expensive organic seed programme. We can fully accept if organic farmers want to use conventional produced seeds, in that case we will provide conventional non-chemical treated seeds, but it should be clear".

Unfortunately, there are still differences between countries in regulations regarding organic crop production. This not only hampers the export of organic products, but also the world-wide supply of organic farmers with high quality seeds and plant material.

#### **Production of high quality organic seeds and support by ISTA.**

Professional organic growers, just like their conventional colleagues, are demanding high quality seeds. The seed industry has taken up the challenges and opportunities for organic seed production by several means, as demonstrated by Jan Velema (Vitalis Biological Seeds). Since no chemicals can be used to control diseases or pests, it is important to find an optimal climate for favourable development of the seed crop. To prevent infec-

**The pamphlet, 'The International Seed Testing Association (ISTA) and Organic Seed Production' can be downloaded from the ISTA Website at [www.seedtest.org](http://www.seedtest.org) or ordered directly from the ISTA Secretariat**

#### **Improving organic seed production and processing**

The conference allowed also the exchange of experience in improving organic seed production and processing, through lectures and posters. Dr. Ruud van den Bulk (PRI, Netherlands) presented how epidemiological studies can be used for setting up a model of critical control points to avoid contamination with *Alternaria radicina* during carrot seed production. Dr. Annegret Schmitt (BBA, Germany) presented a European project on treatments of organic vegetable seeds, giving examples of physical and biological seed

sanitation treatments. The effects of thyme oil for seed sanitation, which I presented myself, received a positive response for being both a component of natural origin and from a crop that can be produced by the farmers. Several examples were provided of dressing seeds with micro-organisms, e.g. the Cerall® treatment of wheat seeds by the Swedish company BioAgri. Interestingly these treatments presented for organic seeds, receive also large attention from the conventional seed sector. Also conventional farmers are interested in reducing the amount of chemicals and seed companies can sell surplus treated seeds still as cattle feed when treated in an environmentally safe way. In this way the challenges for the organic seed sector will provide also possibilities for a more sustainable conventional agricultural production.

#### **More information**

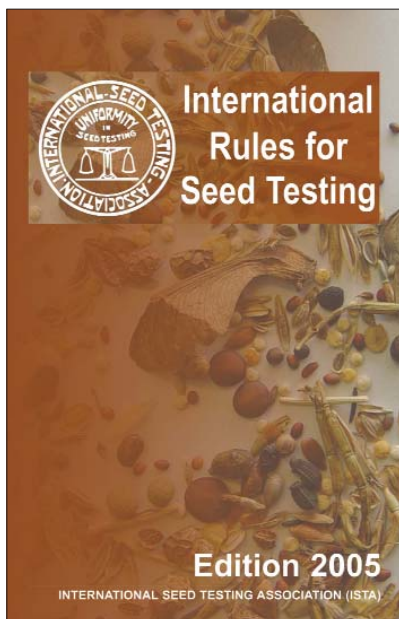
For those that lacked the opportunity to join the conference, there is good news. The very intense and lively discussions and exchange of information, made the organisers promising to continue with the organisation of this conference and we can look forward to a Second World Conference on Organic Seeds.



In the meantime you might read the proceedings (188 pages) that were distributed at the conference and can be ordered through IFOAM for only 24 Euro. ■

For more information contact:  
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Plant Research International  
Wageningen University and Research  
Centre, Netherlands  
e-mail: [steven.groot@wur.nl](mailto:steven.groot@wur.nl)

# International Rules for Seed Testing - 2005 Amendments



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 Rules changes were adopted at the Ordinary Meeting held in Budapest, Hungary, May 20 - 21, 2004. Only the changed pages are included in this package, which is made up of 89 pages. The chapters and pages to be replaced are clearly specified in a table in the preface. The pages from the Edition 2004 that are to be replaced will be no longer valid as of January 1<sup>st</sup>, 2005.

**ISTA Rules Amendments 2005**  
**PRICE: Swiss Francs CHF 102.00**  
 (approx. USD 81.-/EUR 66.-)

**ISTA Rules 2005** (complete set of Rules)  
**PRICE: Swiss Francs CHF 376.00**  
 (approx. USD 298.-/EUR 242.-)

\*Note: The ISTA Rules Amendments 2005 will be released end of October 2004. A complimentary copy will be sent to each ISTA Member as a free service

## CONGRATULATIONS!



We would like to take this opportunity to congratulate Bettina Kahlert and Michael Keller on the birth of their son, Jonas Sasha Aladin Kahlert, who arrived on August 4<sup>th</sup>, 2004.

Bettina is well known to all ISTA Members, especially the Technical Committee Members, as she has been the Head of Technical Committee Administration since January 2002. We expect Bettina to return to the Secretariat early next year.

## Announcement

### 4<sup>th</sup> ISTA Proficiency Test on GMO Testing

ISTA's GMO Task Force is proud to announce the 4<sup>th</sup> ISTA Proficiency Test on GMO Testing, starting in October 2004.

After performing three rounds on *Zea mays* L. there will be a change in the species for the 4<sup>th</sup> round to Soybean (*Glycine max* (L.) Merr).

The aim of the proficiency test is to check the ability of individual laboratories to detect the presence or absence and to quantify the presence of GM seeds in samples of conventional seed of *Glycine max* (L.) Merr.

The results of the proficiency test rounds are intended to be used by the laboratories for their internal performance evaluation. At this stage performance in the tests, based in voluntary participation, will

remain without consequences for the participants. Once GMO testing will become part of the laboratories' scope of accreditation, the results from voluntary proficiency tests on GMO testing, may, upon agreement by the laboratory, be used in the accreditation process. This will in essence speed up the accreditation procedure for laboratories that did participate on a high performance level.

Laboratories interested in participating should please contact the ISTA Secretariat (ista.office@ista.ch or fax +41 1 838 60 01) as soon as possible. More details can be found in the Announcement posted on the ISTA Web at [www.seedtest.org](http://www.seedtest.org)

Participation for members is free of charge (registration fee for non-members: US \$ 200.-/EUR 161.-).

# CALENDAR

## 2004

### November

- 17-19 **ISTA ISTA Workshop on Statistical Aspects of GMO Detection**  
(St Louis, Missouri, United States)
- 22-26 **ISTA/APSA Seed Quality Assessment Training**  
(Hanoi, Vietnam)

## 2005

### April

- 25-28 **ISTA Ordinary Meeting 2005**  
(Bangkok, Thailand)

### May

- 10-13 **5<sup>th</sup> ISTA - SHC Seed Health Symposium**  
(Angers, France)
- 30-2 **ISF Congress**  
(Santiago, Chile)

### August

- 29-2 **7<sup>th</sup> ISTA Seminar on Statistics in Seed Testing**  
(Stuttgart, Germany)



# International Seed Testing Association (ISTA)

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Phone: +41 1 838 6000, Fax: +41 1 838 6001, E-mail: [ista.office@ista.ch](mailto:ista.office@ista.ch)

[www.seedtest.org](http://www.seedtest.org)

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# International Rules for Seed Testing

## Edition 2005

Complete set in two parts (Rules & Annexe to Chapter 7, including Amendments 2005)

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.

**Valid as per January 1<sup>st</sup>, 2005**

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Includes 89 pages to replace current pages in the Rules & Annexe to Chapter 7, Edition 2004

The Rules Amendments were adopted at the Ordinary Meeting held in Budapest, Hungary, May 20 - 21, 2004. Only the changed pages are included in this package, which is made up of 89 pages. The chapters and pages to be replaced are clearly specified in a table in the preface. The pages from the Edition 2004 that are to be replaced will be no longer **valid as of January 1<sup>st</sup>, 2005**.

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\*Note: One free copy of the 2005 Amendments will be delivered to all ISTA Members

# Hotel Reservation Form

*Participants are kindly requested to make their own reservations directly with the Sofitel Central Plaza.*

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**April 25-28, 2005**  
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