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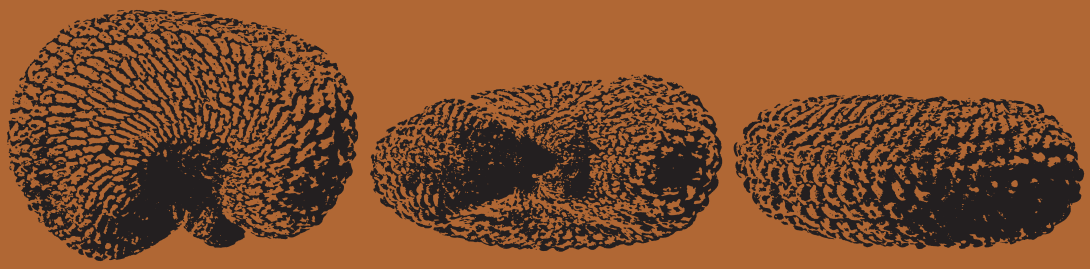

Development of ISTA Purity Analysis and Determination of Other Seeds by Number from 1924 to 2006

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Development of ISTA Purity Analysis and Determination of Other Seeds by Number from 1924 to 2006

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Content

1. Summary

This paper reviews the development of the ISTA purity analysis and determination of other seeds by number from the foundation of ISTA in 1924 to 2006. Focus is on publications and reports which, before the formal validation of Rules proposals, served as the scientific background for changes of the ISTA Rules.

Important steps in the development of the ISTA Rules were: approval of the first ISTA Rules and international certificate (1931); purity tests according to the quicker method (1950); introduction of the three component rule (1974); pure seed descriptions (1976); blowing of grass samples (1966, 1976, 1992); testing of grasses containing multiple seed units (1984); working samples for purity and determination of other seeds by number based on the mass (weights) corresponding to 2,500 seeds (1966) and 25,000 seeds (1990), respectively, and formulation of specific Rules for testing of coated and pelleted seeds (1976, 1985). In the period 1931 to 2006 the number of genera or species included in the Rules has been increased from 41 to 898.

The ongoing Rule changes have improved the uniformity within and between ISTA laboratories and saved time for the analysts. Proficiency (referee) testing, workshops and other training activities as well as use of other supplementary publications have also improved the quality of the analytical work. A large number of colleagues have contributed to the substantial progress in the development and implementation of the purity chapters of the ISTA Rules.

2. Introduction

This paper presents an overview of the development of the International Seed Testing Association (ISTA) purity analysis and determination of other seeds by number from the foundation of ISTA to the present time. Before a validation programme became introduced (Hampton 2005), the published reports and the results of the testing activities in the working groups served as the scientific background for the changes of the ISTA Rules.

Papers on the background and history of the International Seed Testing Association (ISTA) have been published by Wold (1975, 1996) and Steiner (1998, 1999, 2000). The progress in the testing of germination has been summarized by Klitgård (2003) and the activities of the ISTA Plant Disease Committee have been reviewed by Mathur and Jørgensen (2003).

3. The Purity Committee

3.1 Committees prior to the Purity Committee

Prior to forming the ISTA Purity Committee in 1956, the development of the Rules for purity and determination of other seeds by number, as well as other technical and scientific work related to these tests, was from 1924 to 1950 dealt with by the Research Committee for Countries with Temperate Climate and the Research Committee for Countries with Warm Climate. From 1953-1956 the work was coordinated by the Rules Committee.

From 1924 to around 1950 the following technical committees were working on subjects related to purity analysis and determination of other seeds by number: Provenance Determination, Dodder Committee and Beet Sub-Committee. Other committees were formed later: Research Committee for Forestry Seed (1928-1971), Education Committee (1928-1931), Ad hoc Committee for Studying the SM (stronger method) and QM (quicker method) (1937-1950), ad hoc Certificate Committee (1956-1959), Pelleted Seed Committee (1968-1987) and Flower Seed Committee (1971-). Details of year of foundation, the chairmen, and who continued the tasks of discontinued committees have been published by Wold (1975, 1996).

3.2 Members and chairpersons of the Purity Committee from 1956-2006

In total 80 individuals from 28 countries have contributed to the development and interpretation of the ISTA Rules (table 1). Among those 47 were from Europe, 10 from Australia/New Zealand, 10 from North America, 4 from Africa, 2 from South America, 1 from Asia, and for 6 persons no information on country was given in the Committee report.

Table 1. Members and chairpersons of the Purity Committee from 1956 to 2006. The list is arranged according to the year the members joined the committee.

Periods	Members	Country	Chair-persons
1956-1959	Mr. E. Debney	*)	
1956-1962	Mr. H. Esbo	Sweden	Chair 1956-1962
1956-1980	Dr. Leroy E. Everson	USA**)	Chair 1962-1977
1956-1959	Mr. R.A. Ingalls	*)	
1956-1959	Mr. M. Ingold	*)	
1956-1959	Mr. A.A.M. Lotfy	*)	
1956-1959	Ms. Amy Myers	*)	
1956-1959	Mr. E.W. Sundermeyer	*)	
1956-1965	Mr. J.R. Thomson	United Kingdom	
1956-1968	Mr. Arne Wold	Norway	
1962-1980	Mr. J. Bartz	Poland	
1962-1965	Mr. T.F. Cuddy	Canada	
1962-1974	Ms. Gwen Easton	Australia	
1962-1971	Mr. D.B. MacKay	United Kingdom	
1962-1968	Mr. Hubert Barde	Switzerland	
1962-1965	Mr. B. Nilsson	Sweden	
1962-1977	Mr. Marius Olesen	Denmark	
1962-1965	Dr. H.H. Schmidt	Germany	
1965-1971	Dr. J.E Boeke	The Netherlands	
1965-1977	Dr. M.A. Filimonov	Russia	
1965-1986	Mr. Olle Landenmark	Sweden	Chair 1977-1986
1965-1968	Mr. A.C. Peel	Australia	
1965-1971	Mr. C.B.W. Rogers	Canada	
1965-1980	Dr. B. Schmidt	Germany	
1965-1974	Mr. R. Seaton	United Kingdom	
1968-1974	Ms. D.E.M. Johnson	New Zealand	
1968-1971	Mr. Bernard M. Leese	USA	
1968-1974	Mr. E.T. Prodonoff	Australia	
1971-1992	Mr. T. J. Arthur	United Kingdom	
1971-1987	Ms. E.M. Felfoldi	Australia	Vice-chair 1977-1987

Table 1. *Continued*

Periods	Members	Country	Chair-persons
1971-1982	Mr. K.B. Hanssen	Rhodesia, Zimbabwe	
1971-1974	Mr. R. Lopez de Haro	Spain	
1971-1977	Ms. E.F. Wiseman	USA	
1974-1980	Ms. R. Berdejo	Spain	
1974-1995	Mr. Jack E. Butler	Australia	
1974-1983	Mr. S.R. Cooper	United Kingdom	
1974-1977	Mr. F.I.R. da Silva	Portugal	
1974-1991	Mr. M.S. Dhaliwal	Canada	
1974-1980	Ms. N. Döslüoglu	Turkey	
1974-1977	Mr. M. Kerguelen	France	
1974-1989	Ms. D.E.M. Meech	New Zealand	
1974-1980	Mr. Nicu Pana Pompei	Roumania	
1974-1983	Ms. E. Roberts	South Africa	
1974-1977	Ms. Krystyna Tomczyk	Poland	
1974-1977	Ms. G.R. Vennell	Australia	
1977-1980	Ms. M.E. Grilo	Portugal	
1977-2001	Dr. Hans Arne Jensen	Denmark	Chair 1986-1998
1977-1983	Mr. J. Machanicek	Czechoslovakia	
1980-1998	Ir. W.Joost van der Burg	The Netherlands	Vice-chair 1989-1998
1980-1995	Dr. Arnold Larsen	USA	
1980-1995	Dr. Norbert Leist	Germany	
1980-1986	Mr. G. Rytko	Poland	
1983-1998	Dr. Peter Gosling	United Kingdom	
1983-1998	Mr. André Maréchal	France	
1983-1998	Ms. Pamela J. Strauss	South Africa	
1986-1992	Dr. John E. Ferguson	Colombia	
1986-1995	Mr. Ulf Kjellström	Sweden	
1986-1992	Ms. Janina Mazgaj	Poland	
1989-1998	Ms. Angela M. Finnerty	New Zealand	
1992-2001	Mr. Didier Demilly	France	Vice-chair 1998-2001
1992-1998	Mr. Doug B. Ashton	Canada	

Table 1. *Continued*

Periods	Members	Country	Chair-persons
1992-2006	Ms. Monica Moreno	Argentine	Chair 1998-2001
1995-2001	Mr. Olla Kristian Dille	Norway	
1995-2006	Dr. Axel Göritz	Germany	
1995-2006	Ms. Deborah J. Meyer	USA	
1995-2001	Dr. Elzbieta Maluszynska	Poland	
1995-2001	Mr. Miguel Oliveras	Sweden	
1998-2004	Mr. Ken Allison	Canada	Chair 2001-2004
1998-2001	Ms. Ansie de Vries	South Africa	
1998-2006	Dr. Steve Jones	United Kingdom	
1998-2003	Ms. Anny van Piljen	The Netherlands	
1998-2001	Dr. Kirsten Thomsen	Denmark	
1998-2006	Ms. Zita Ripka	Hungary	
2001-2003	Dr. Heidi Elberling	Denmark	
2001-2006	Dr. Maria R. Mannino	France	Vice-chair 2001-2003. Chair 2004-
2001-2006	Mr. Andreas Ratzenboeck	Austria	
2001-2006	Dr. Jane Taylor	United Kingdom	Vice-chair 2004-2006
2004-2006	Dr. Fabio Ferrari	Italy	
2004-2006	Dr. S.C.Aswath Narayana	India	
2004-2006	Ms. Gerarda de Boer	The Netherlands	

*) Information on the country not given in the committee report. **) Obituary, see Jensen (2005)

4. Function of the Purity Committee

4.1 Planning of the working programme

The tasks dealt with in the Purity Committee were formulated in the *Programme* and later as *Terms of reference*.

An updated set of *Terms of Reference* was prepared and discussed in the Purity Committee both by correspondence prior to and at meetings during the Congress. They were based on the progress of the work in the Purity Committee and problems raised either by members of the Purity Committee, the Executive Committee or other groups.

The adjusted programme was finally approved by the Executive Committee. Sometimes new urgent subjects came up between Congresses. Such questions were usually dealt with by ad hoc groups in the Purity Committee and the subjects were, if needed, included in the working programme for the following period.

The progress and the problems related to the working programme were reported at the Inter Convention Meetings with the Executive Committee, held 1 to 1½ years before the next Congress.

Before the Congress the tasks dealt with and the progress in the work on various subjects was summarized in the *Report from the Purity Committee*. The report, including proposals for changes of the ISTA Rules, was then discussed during meetings of the technical committees at the ISTA Congress, and the final report (sometimes with minor adjustments of the rule proposals) was presented and voted on during the ordinary meetings of the Congress.

The reports were published in the Congress reports (Esbo 1960, 1962; Everson 1965, 1969, 1972, 1975, 1978; Landenmark 1981, 1984, 1987; Jensen 1989, 1992, 1995, 1998a; Moreno 2001; Allison 2002, 2003, 2004; Mannino 2005, 2006).

4.2 Terms of reference

The Programme or Terms of Reference consisted of:

4.2.1 Long term projects

Some of the long term projects have been part of the terms of reference for most of the period. Examples are:

- Revision of the ISTA Rules (specific the Pure Seed Definitions)
- Testing of tropical and subtropical seeds
- Technical aids for purity analysis and determination of other seeds by number (e.g. optical aids, blowing).

4.2.2 Requests from the Executive Committee

Since the foundation of ISTA until around 1990 most ISTA seed laboratories received a substantial financial support both for the cost of running the seed laboratories and for international activities in ISTA and similar organisations. Due to the decreasing government support and privatisation of seed testing, the voluntary work within the technical committees of ISTA became more and more limited. In some countries the economic changes began before 1990, in other countries mainly after 1990; accordingly, a distinction is made between *before and after 1990* in the following.

Before 1990: The Executive Committee had in general only limited requests to the Purity Committee; examples are:

- Introduction of 'quicker method' as the only method allowed in purity analysis (requests from the seed trade and seed laboratories from around 1950)
- Harmonisation between the ISTA and the AOSA Rules (requests from governments and seed trade in the EU and in the USA, from 1988 onwards).

After 1990: The restricted funds available for ISTA activities and the reduced staff at official ISTA seed testing laboratories resulted in increased requests from the Executive

Committee to the Purity Committee and other technical committees to concentrate on fewer subjects. The importance of specific problems was emphasized through meetings with governmental representatives, international organisations and the seed trade. Examples are:

- Quality assurance (1998 to the present time)
- Seed count (2001 to the present time)
- Seed mixtures (1995 to the present time).

4.2.3 *Requests from the Seed Trade*

- Specification of other crop seeds and weed seeds on the ISTA certificate after introduction of the three component rule (1974)
- Blowing of small seeded varieties of Poa pratensis (2003).

4.2.4 *Requests from the seed laboratories (in order to save time during analyses or improvement of the reproducibility of the analyses)*

- Blowing of grasses (1966, 1992)
- Testing of multiple seed units in grasses (1979, 1981, 1984)
- Allowing working samples on 2,500/25,000 seeds for purity and number count tests (1966, 1990)
- Allowing purity and number count tests before chemical treatment (1992).

4.2.5 *Subjects included in the working programme based on either personal interest of the committee members or research going on at a particular seed testing station*

- Identification of seeds from a specific area
- Determination of other seeds by number by image analyses (seed scanners).

Some of the minor tasks in the *terms of reference* were dealt with by correspondence within the Purity Committee and perhaps discussed during Workshops and Congresses. Most of the major tasks, however, required either specific technical developments or try-outs of various options before a Rules change could be proposed.

4.3 Working groups

For a number of tasks dealt with in the Purity Committee it was realized that formation of specialised working groups was necessary. The groups included members of the Purity Committee as well as individuals with no other affiliation to the committee.

The working groups gained from contributions from a larger group of specialists, and the members of the working groups obtained valuable international experience during this work.

An overview of the tasks dealt with by working groups through the years is given in table 2. It presents in chronological order the year of establishment and if appropriate the year of closing of the working group, the name of the group and the chairpersons responsible for the work.

The reporting from the working groups and changes of the ISTA Rules are presented later in the report, see for example 5.7 Pure Seed Definition and 5.8 Multiple Seed Units.

Table 2. Working groups established by the Purity Committee.

Period	Name of working group	Chairperson
1965-1968	Dactylis glomerata purity analysis	L.E. Everson, USA 1965-1968
1965-1968	Flower seed analyses	J.E. Boeke, The Netherlands 1965-1968
1965-1968	Separation of Poa trivialis/Poa pratensis	M. Olesen, Denmark 1965-1968
1965-1971	Beta seed analysis	M. Olesen, Denmark 1965-1971
1965-1974	Weed seed evaluation	A. Wold, Norway 1965-1968 R. Seaton, United Kingdom 1968-1974
1965-2006	Pure seed definitions (Crop seed definitions)	D.B. MacKay, United Kingdom 1965-1968 T.J. Arthur, United Kingdom 1971-1977 E.M. Felfoldi, Australia 1977-1987 W.J. van der Burg, The Netherlands 1987-1998 Steve Jones, United Kingdom 1998-2006
1965-2004	Tropical and subtropical seeds	A.C. Peel, Australia 1965-1968 Gwen Easton, Australia 1968-1974 G.R. Vennell, Australia 1974-1977 K.B. Hanssen Zimbabwe 1977-1982 A. Larsen, USA 1983-1986 J. Butler, Australia 1986-1992 Monica Moreno, Argentina 1992-2004
1968-1977	Rules for purity analysis (e. g. classification of weed seeds, reporting other crop seeds and weed seeds as other seeds, definitions of pure seeds (PSD))	D.B. MacKay, United Kingdom 1968-1971 R. Seaton United Kingdom 1971-1974 E.F. Wiseman, USA 1974-1977
1968-1971	Optical aids for purity analysis	J.E. Boeke, The Netherlands 1968-1971
1968-2005	Blowing procedure for grasses	L.E. Everson, USA 1968-1974 S.R. Cooper, United Kingdom 1974-1977 L.E. Everson, USA 1977-1980 J. Butler, Australia 1980-1983 W. Joost van der Burg, The Netherlands 1995-1998 Anny van Piljen, The Netherlands 1998-2004 Gerarda de Boer, The Netherlands 2004-2005
1968-1983	Multiple seed units in grasses	M. Olesen, Denmark 1968-1977 Hans Arne Jensen, Denmark 1977-1983
1971-1977	Classification of immature or sterile florets, fruits or seeds	O. Landenmark, Sweden 1971-1974 E. Felfoldi, Australia 1974-1977
1974-1977	Calibration samples	L.E. Everson, USA 1974-1977
1977-1983	Mechanical aids in purity and number count testing	O. Landenmark, Sweden 1977-1983
1977-2006	Identification of seeds, bibliography on seed morphology	Hans Arne Jensen, Denmark 1977-2001 Heidi Elberling, Denmark 2001-2004 The Purity Committee 2004-2006

Table 2. *Continued*

Period	Name of working group	Chairperson
1983-2004	Referee testing committee liaison	W. Joost van der Burg, The Netherlands 1983-1986 Members of the Purity Committee 1986-1992 Didier Demilly, France 1992-2001 Ken Allison, Canada 2001-2004
1985-1992	Avena species, fatuoides, hybrids (Identification and evaluation)	Norbert Leist, Germany 1980-1992
1986-1989	Calculation of purity results	W. Joost van der Burg, The Netherlands 1986-1989
1989-2001	Image analyses, blowers and other mechanical devices	Ulf Kjellström, Sweden 1989-1992 Members of the Purity Committee 1992-1998 Axel Göritz, Germany 1998-2001
1992-1995	Testing of Bromus catharticus and other Bromus species according to the MSU method	Angela M. Finerty, New Zealand 1992-1995
1995-2001	Seed count	Miquel Oliveras, Sweden 1995-2001
1995-2001	Quality assurance	Didier Demilly, France 1995-1998 Ola Christian Dille, Norway 1998-2001
1998-2006	Seed mixtures	Ola Christian Dille, Norway 1998-2001 The Purity Committee 2001-2006
2001-2004	ISTA website liaison	Ken Allison, Canada 2001-2004
2001-2006	Workshops development and organisation, training	Maria R. Mannino, France 2001-2004 Ken Allison, Canada 2004-2005
2004-2006	ISTA Rules modifications	Maria Rosario Mannino, France 2004-2006.

A summary of the activities in the working groups were usually included in the report from the Purity Committee, while the final outcome of the work were in several cases published by the leader of the working group in specific reports (Boeke 1969; Butler 1983; Easton 1972; 1975a; 1975b; Felfoldi 1978a; Jensen 1981; 1984a; 1998b; Leist 1981; 1992; MacKay1968¸ Olesen 1969; Peel 1969; Seaton 1975; Vennell 1972, 1975, 1978; Wold 1968).

4.4 Contacts with the ISTA Secretariat

The ISTA Secretariat and the Purity Committee worked closely together throughout the whole period. The Secretariat facilitated information on meetings, forwarded questions from ISTA members, helped with deadlines for replies, Rule changes and publication of Rules and handbooks etc. Valuable assistance was, furthermore, received from the ISTA Secretariat in connection with Congresses, Workshops, and other meetings.

4.5 Correspondence

Members of the Purity Committee and the working groups kept in touch mainly through correspondence (by air mail and later by e-mail), as the possibilities for personal meetings between the members were limited. The frequency of the correspondence from chairmen of the Purity Committee and its working groups and replies from the members varied

through the period between Congresses. The highest activity was always just before and after the Congresses.

It now seems normal to have daily contact by e-mail, but until around 1990 letters were typed and posted as air mail to various parts of the world. At least 1-2 months should be reserved from a letter was shipped until the answers were received from all members of the Purity Committee.

4.6 Meetings

During Congresses and Workshops separate meetings were held for the Purity Committee. The meetings were most often open to all participants. The aim was to inform about the ongoing work and particularly to inform about planned changes of the ISTA Rules. Reactions from ISTA members were important for making the changes of the ISTA Rules understood by all participants, and for writing the ISTA Rules as user friendly as possible.

5. **Development of the ISTA Rules**

5.1 The first ISTA Rules

When the first ISTA Rules were presented by Franck (1931), the Netherlands, the consequences for the member laboratories were:

- *The International Rules are exclusively … for the seed testing for international usage*
- *Each station … is not bound to issue International Certificates*
- *When an International Certificate is used, it must be based on an examination according to the International Rules.*

5.2 Size of working samples

5.2.1 Purity test

The first ISTA Rules contained minimum mass (weights) for 41 species or groups of species. These figures were established by combining the national Rules of several countries, but apparently without any attempt to reach a certain number of seeds for the purity test.

The ISTA Rules 2005 contain minimum mass (weights) for 898 taxa (species or genera), of which 316 are agricultural and vegetable plants, 230 are trees and shrubs and 352 are flower, spice, herb and medicinal plants. The year of introduction into the ISTA Rules of the agricultural and vegetable plants has been listed by Klitgård (2003). The main part of the trees and shrubs was included in the 1966-Rules, and an essential part of the flower, spice, herb and medicinal plants were adopted in the ISTA Rules at the Congress in 1977.

Since the beginning of ISTA it was known that the size of the working sample was a compromise between the working hours involved in performing the test and the ability to reproduce the results (see table 3).

Table 3. Minimum mass (weights) (in g) for purity analysis of selected species in the ISTA Rules of 1931, 1956, 1966 and 2005.

Species	1931*	1931**	1956**	1966**	2005**
Agrostis stolonifera	0.5	1	0.5	0.5	0.25
Allium cepa	5	10	10	8	8
Allium porrum	5	10	10	7	7
Anethum graveolens	2	4	5	4	4
Avena sativa	50	100	100	120	120
Cucumis sativus	25	50	100	70	70
Dactylis glomerata	1	2	2	3	3
Daucus carota	1	2	5	3	3
Festuca ovina	1	2	2	3	2.5
Festuca rubra	1	2	2	3	3
Lactuca sativa	2	4	5	3	3
Linum usitatissimum	5	10	10	15	15
Lolium perenne	3	6	5	6	6
Lupinus angustifolius	100	200	500	450	450
Pisum sativum	100	200	500	900	900
Poa pratensis	0.5	1	1	1	1
Trifolium repens	2	4	2	2	2
Zea mays	200	400	500	900	900

** Half working samples ** whole working samples*

Table 3 illustrates that for ten of the selected species, the working samples examined in 2005 were bigger than in 1931, for three species it were the same and for five species the size of the sample was reduced.

In the ISTA Rules of 1931 it was obligatory to examine two replicates in the purity test. Since 1962 this became an option but still some laboratories test two working samples as part of their internal quality procedures.

The tolerances calculated by Miles (1963) were based on an examination of a working sample corresponding to 2,300 chaffy seeds and 2,750 non-chaffy seeds (see 5.3). The ISTA Congress in 1965 did not find it necessary to distinguish between the two types of seeds, and it was decided that the purity examinations and the tolerances should be based on a sample corresponding to 2,500 seeds (Thomson 1965).

The size of the working samples was from time to time discussed. In the Scandinavian Rules for seed testing of 1912 the size of the working sample was based on a working sample of 2,000 seeds.

During the ISTA Workshop in 1973 half working samples (1,376 from Finland and 1,273 from Denmark) were compared with the ISTA tolerances of 1963. The results showed that the laboratories in Finland and Denmark could use the ISTA tolerances without problems, even though the national examinations were performed on samples corresponding to 2,000 and not 2,500 seeds (Jensen 1974).

5.2.2 Determination of the number of other seeds, weed seeds or noxious seeds
The ISTA Rules of 1931 prescribed that: *Determination of the total number of weed seeds or of the number of noxious seeds must be made on two analyses, each of the quantity of at least 5 times the amounts given in the above table for purity analysis, except when some other prescription is given (e. g. for dodder).*

For *Anethum graveolens* the prescribed mass (weight) in 1931 was 2g for a half purity working sample. The minimum working sample for count of weed seeds was then: 2g x 2 analyses x 5 = 20g or half the amount prescribed in the ISTA Rules of 2005. Other examples on the changes in the amounts of seeds examined for number count tests from 1931 to 2005 are given in table 4.

Table 4. The mass (weight) required to be examined for determination of other seeds by number according to the ISTA Rules of 1931, 1956, 1966 and 2005.

	1931	**1956**	**1966**	**2005**
Agrostis stolonifera	5	25	5	2.5
Allium cepa	50	100	80	80
Allium porrum	50	100	70	70
Anethum graveolens	20	50	40	40
Avena sativa	500	500	1,000	1,000
Cucumis sativus	250	500	150	1,000
Dactylis glomerata	10	50	30	30
Daucus carota	10	50	30	30
Festuca ovina	10	50	30	25
Festuca rubra	10	50	30	30
Lactuca sativa	20	50	30	30
Linum usitatissimum	50	100	150	150
Lolium perenne	30	50	60	60
Lupinus angustifolius	1,000	1,000	1,000	1,000
Pisum sativum	1,000	1,000	1,000	1,000
Poa pratensis	5	25	5	5
Trifolium repens	20	50	20	20
Zea mays	2,000	1,000	1,000	1,000

The requirement of testing 10 times the amount examined for purity with a maximum of 1,000g was introduced into the ISTA Rules in 1966.

The ISTA Rules of 1931 specified that the examination for *Cuscuta* (dodder) in seed samples of *Trifolium pratense, Medicago sativa* and other seeds of similar size should be

performed in 100g. For *Trifolium repens, Trifolium hybridum* and *Phleum pratense*, the examination for *Cuscuta* should be performed in 50*g*. If a high number of *Cuscuta* seeds were found, the amount examined could be reduced.

Since 1990 it was included in the Rules that the working sample for number count tests shall be: either a weight estimated to contain 25,000 seed units or not less than the weight prescribed in table 2A, part I, column 5.

The 25,000 seed Rule allowed the laboratories to make adjustments according to the considerable differences in thousand seed weight between varieties of several grass species. At least in the Netherlands and Denmark, countries in which large number of grass samples is tested, official tables of the seed mass (weights) of small seeded varieties were prepared. The 1,000 seed weight was used for the calculation of the required weight of the working sample for determination of other seeds by number.

Tables with seed mass (weights) of different varieties of *Lolium perenne* and *Festuca ovina* were presented at the Budapest Workshop 1997 (Jensen 2000). As an illustration of this, the mass of 25,000 seeds of the small-seeded *Lolium perenne* variety Loretta was only 33g, whereas the standard working sample according to table 2A, column 5 was 60g in 2005. This shows that for certain varieties considerable time can be saved by using the 25,000 seed rule for determination of other seeds by number.

5.3 Tolerances

The accuracy of purity testing was of concern from the beginning of seed testing within ISTA, and a formula for calculating the allowed tolerance between two tests was presented by Munn (1931). In the equations below, p represents the 'greater part' and q the 'lesser part' (e. g. % pure seed and the sum of % inert matter, % other crop seeds and % weed seeds, respectively).

The equation for pure seed was:

$$\text{Tolerance} = 0.6 + 20\% \text{ of } p \times q \div 100$$

The equation for other crop seed, weed seed and inert matter was:

$$\text{Tolerance} = 0.2 + 20\% \text{ of } p \times q \div 100$$

Munn (1932) published tables for the purity test. Leggatt (1932) criticised that the equation did not take into account that *the larger the sample, numerically, the smaller the expected variation*, and pointed out that it would be more correct to use the equation for calculation of the standard deviation. In other words, if the size of the working sample for purity was increased, a smaller variation between tests could be expected.

Leggatt published descriptions of the experimental error in seed analyses, including the normal -, binomial - and the Poisson distributions, and a theoretical study of a purity tolerance with special reference to pure seed during the following years (Leggatt 1934, 1935a, 1935b, 1936).

Leggatt (1933) also examined the incidence of weed seeds in duplicate analyses, and analysed the data by use of the binomial distribution. The sampling method used in Canada provided random samples except for a few cases, for instance when the seed lots contained *Plantago rugelii* and *Plantago major*, of which the seeds occur as clusters in the seed lots.

Stahl (1937) used the mean standard deviation for establishment of tolerances (latitudes) for two half working samples in the purity tests and for calculation of tolerances for determination of weed seed contents.

A study on the occurrence of *Rumex crispus* in replicate analyses of *Dactylis glomerata*, which included different methods of sampling, was published by Woodbridge (1935), and Przyborowski (1938) used of the Poisson distribution to examine the errors due to insufficient size of clover samples tested for dodder.

In the ISTA Rules of 1953, the tolerances from 1931 were expanded to include chaffy grasses. For purity, the following was added to the regular tolerances:

$$\text{Regular tolerance} \times (100\text{-value}) \div 100$$

If per cent pure seed, for instance, was 91.4% and the tolerance 2.17, the tolerance for chaffy grasses would then be 2.36%.

A revised set of tolerances was calculated by Miles and introduced into the ISTA Rules in 1962. The tolerances were based on results from seed testing, obtained in Canada and USA and calculated by use of the binomial distribution (Miles *et al.,* 1960; Miles 1963). In connection with this change Miranda (1962) and Thomson (1963) discussed the general principles for the revised purity and germination tolerances. The tolerances, calculated by Miles (1963), are still in use in the ISTA Rules of 2006.

5.4 From stronger method to quicker method

Before ISTA was founded in 1924 two methods were in use for purity analysis in Europe: *the Continental method* and *the Irish method* (also called *the English method).*

By *the Continental method*, all seeds were examined first by the naked eye or using magnification and then under reflected light. Under the reflected light:" *all empty seeds are detected and reckoned as impurities. Only the full seeds are admitted to the determination of the germination power. According to the Irish method the examination by translucent light is not used and consequently the half empty and empty seeds are tested for germinating power"* (Bruijning 1922).

The subject was intensively discussed during the Congresses at Copenhagen (1921), Cambridge (1924) and in Rome (1928). During the Congress at Wageningen (1931) it was not possible to obtain an agreement about a preferred method, and both *the Stronger method (*corresponding to the *Continental method)*, and *the Quicker method* (a modified form of *the Irish method* from North America)*,* were included in the first ISTA Rules of 1931.

The stronger method required that the seeds, classified as pure seed, did not have any damage on vital parts of the seed. Undamaged seeds were, accordingly, considered to be able to germinate. As guidance, drawings of clover seeds with various degrees of damage were included in the Rules.

The quicker method was based on the assumption that all pieces of seeds of the species tested, with a size of more than half the original size, should be classified as pure seed. The evaluation of whether the seeds were able to germinate was supposed to be determined in the germination test.

The stronger method was tested by a comparison of results from identical clover samples, containing damaged seeds at eight seed testing laboratories. The results disclosed a difference of 6.6%. The lack of uniformity was mainly due to various interpretations of the descriptions of damages seeds (Saulescu and Szopos 1938).

At the ISTA seed Congress in 1937 Franck (1938) gave an introduction to purity testing according to the *stronger method* and *the quicker method,* and Wright (1938) explained that the Canadians found the uniformity between tests improved and the time required for analysis reduced when using the *quicker method.*

The discussion was interrupted by the 2nd world war, but at the first ISTA Congress after the war Franck (1950) recommended once more the *quicker method* as the only allowed purity method in the ISTA Rules. By this time the *quicker method* was already in use both in USA, Canada and in the Scandinavian countries.

ISTA's two methods for purity testing became an increasing inconvenience for the seed trade, and the Russian delegation in the International Organization for Standardization (ISO) raised the question whether ISO should go into standardization of seed testing and that they should begin with purity. This question was submitted via the ISTA member countries standardization organizations to the ISTA laboratories and to the ISTA Secretariat. It was taken as a serious warning that decisions on formulation of the Rules could move to other organisations, and the ISTA Congress in 1950 finally decided that the *stronger method* should be deleted and the *quicker method* should be the only method for purity analysis in the ISTA Rules (Franck 1950).

Purity tests of some of the tropical grass seed species are very time consuming. Accordingly, for *Chloris gayana,* the *Irish method* was compared with the ISTA purity methods for determination of pure live seed (Loch and Mulder 1987). The *Irish method* defines pure seed as any intact seed unit irrespective of whether a caryopsis is present or not. The ISTA purity method (PSD 42) requires that pure seed of *Chloris gayana* must contain a caryopsis. The comparison showed that the *Irish method* underestimated the content of Pure Live Seed by up to 10-20 percentage points.

5.5 Evaluation of weed seeds

A working group with the task to examine evaluation of weed seeds on the same basis as crop seeds was founded at the ISTA seed Congress in Munich 1965. Wold (1968) and Seaton (1975) reported that most of the 25 laboratories taking part in the investigation found that evaluation of weed seeds on the same basis as crop seeds had little effect on

the final results, and that applying the half seed rule to crop seeds as well as weed seeds would be an advantage to the seed analysts and increase uniformity of testing.

The half seed rule for weed species and other crop seeds was accepted at the ISTA Congress in 1974.

5.6 The three component rule

At the Congress in 1974 the three component rule, i.e. combining other crop seeds and weed seeds into one fraction *other seeds,* was accepted, as it was impossible to obtain common agreement on a world wide basis on the classification of the species found in the seed samples into either crop species or weed species.

This change of the ISTA Rules was very much against the wishes of the seed trade. After the Congress an attempt was made to persuade ISTA to arrange a new vote by post, to reverse the decision of 1974. This, however, was refused by the Executive Committee, as the technical reasons for the change of the ISTA Rules were still valid.

In the welcome speech to the ISTA Congress in 1977 (*Seed Science and Technology,* **6**, 39) S.F. Rollin, president of ISTA, said: *As you all know, the most vexing problem we have had to deal with for the past three years has been the adoption of the three-component rule for purity testing. Despite being forewarned and researched and reported and discussed for several years, when the three-component rule was finally approved at the Warsaw meeting to go into effect last year, the FIS recorded its opposition in no uncertain terms. It continues to do so.*

Seaton (1978) summarized the ongoing discussion on the three component rule. Arguments in favour of the three component rule included that seed is moving internationally and it was not possible to obtain international agreement on classification into weeds and crops. Replies from 37 countries taking part in a survey submitted to all ISTA member countries showed that of the 234 agricultural and horticultural species included in table 2A, only 27 were consistently classified as crop species. The remaining 207 species could be classified as either crop species or weed species (Seaton and Bjørnstad 1978).

Arguments against the three component rule mainly came from the seed trade, e.g.: "*Use of the three component rule will decrease the usefulness of the international certificates. One can convert a four component report into a three component report but the reverse is not possible*."

During the discussion of the paper Classification of Seeds at the 1977 Congress (Seaton and Bjørnstad 1978, p. 125-136) R. Jacobsen, a representative of the Seed Trade Union FIS (predecessor of ISF), said: "*The Warsaw decision on three components has met considerable opposition from the great majority of FIS members and you know that we have opposed this change from the very beginning. Therefore FIS can only propose to ISTA to change back to the four components…On behalf of our 50 member-countries I make a plea to you to consider this proposal favourably. As the users of the ISTA certificates we believe that we are entitled to this."*

Many arguments in favour but also against the three component rule were expressed. Finally, it was agreed as a compromise that the content of other seeds, reported on the ISTA certificate, could be - as a specification - divided into other crop species and weed species according to the definitions at the issuing laboratories or legal requirements. By

accepting this change the serious conflict between FIS (The Seed Trade) and ISTA was solved. The specification into other crop seeds and weed seeds on the ISTA Certificates is still in use.

5.7 Pure seed definitions (PSD)

A working group was appointed by the Purity Committee in 1965 to prepare botanical descriptions of structures classified as *pure seeds* or *other crop seeds* in the purity analysis. A report from the working group was presented at the Congress in 1968 (MacKay 1968). The Pure Seed Definitions (PSD) was included into the ISTA Rules of 1976.

The preparation of PSD for the ISTA Rules was continued in a working group, chaired by Elisabeth M. Felfoldi, and later by W. Joost van der Burg and Steve Jones. An important step forward was the publication of the Handbook of Pure Seed Definitions (Felfoldi 1978b). A revised 2nd edition was published in 1987. The illustrations, especially of the tropical grasses with complicated morphological structures, were a considerable help in the analyses of those grasses.

A similar handbook on PSD for tree and shrub seeds was compiled by W. Joost van der Burg (1991). A revised PSD handbook, including all PSDs in the ISTA Rules, is under preparation in the Purity Committee (Jones *et al.,* 2005).

Informative line drawings of the morphological structure of a number of grass seeds can be found in the article "Understanding grass family seed units" (Meyer 1998).

A large number of studies examined the grass inflorescences, before PSDs could be defined for this group. Madsen (1960), for example, investigated the ratio between pure seed and inert matter in multiple florets of *Dactylis glomerata;* the classification of florets, based on the size of the caryopsis, was studied for the same species by Seaton (1968) and Felfoldi (1975).

Pure seed of *Lolium perenne, Festuca rubra, and Festuca ovina* var. *duriuscula* should contain an obvious caryopsis containing endosperm (Olesen 1968b). Measurement of the length of palea and the length of caryopsis followed by a germination test demonstrated that for all seeds able to germinate, the length of caryopsis was at least 1/3 the length of palea. The introduction of the 1/3 rule for *Lolium perenne, Festuca rubra, and Festuca ovina* var. *duriuscula,* and for *Agropyron repens* (Olesen and Langkilde 1965, Olesen 1968a) was an important step towards a uniform and time saving classification of these grass seeds.

Some of the pure seed definitions (PSD) were modified in order to reach harmonisation with the AOSA Rules. For Fabaceae (PSD 10), separated cotyledons were since 1992 regarded as inert matter, irrespective of whether or not the radicle-plumule axis and /or more than half of the testa may be attached (Jensen 1992). This definition is still in use for seeds of Fabaceae.

5.8 Multiple seed units (MSU) and seed with appendages

The ISTA Rules of 1931 prescribed for purity analyses that in grass seeds containing many-flowered spikelets, the sterile flowers are separated and counted as inert matter. Many-flowered spikelets were later named multiple seed units (MSU).

Purity analysis of grasses with a high number of multiple seed units (MSU) containing both fertile and sterile florets was time consuming, as the seed units should be separated by a knife, and only the units containing a caryopsis were classified as pure seeds, whereas the sterile florets were part of the inert matter fraction.

The ISTA Rules of 1956 specified that sterile florets attached to a fertile floret shall be left attached and included in the pure seed fraction for *Chloris gayana, Arrhenatherum elatior, Dactylis glomerata* and *Poa species*. MSU's in *Dactylis glomerata,* containing at least one caryopsis, should be divided into four –fifths of the weight, which should be added to pure seed and one-fifth should be added to the inert matter (Seiferle and Porter 1937; West 1952; Madsen 1960).

In 1971 a special time saving clause was added to the Rules for *Festuca rubra* and *F. ovina:* Attached sterile florets, which did not extend to the tip of the fertile florets, must not be removed from the fertile florets and should be considered part of pure seed (Olesen 1968b).

Separating the multiple seed units with attached sterile florets extending to beyond the tip of the fertile florets was still a time consuming work. Therefore, a working group was set up in 1968 to deal with the examination of multiple seed units, especially in *Festuca* and *Dactylis*, and to prepare proposals for revision of the Rules.

Laboratory experiments demonstrated that due to variation between seed samples in content of inert material in the multiple seed units it was impossible to find either a common factor, estimating the ratio between pure seeds and inert matter, or to find a mathematical equation for calculation of the content of pure seed in the MSU fraction of those grasses. Therefore, it was proposed for *Festuca* and *Dactylis* that MSU should be included in the pure seed fraction, and the content of MSU should only be reported on the certificate if representing 1% or more of the sample (Jensen 1979f, 1981).

The proposal was included in the ISTA Rules in 1980 and slightly modified in 1983 (Jensen 1984a). In 1995 it was accepted that *Lolium* should be reported in the same way as *Festuca* and *Dactylis*.

The adoption of the testing multiple seed units in *Festuca* and *Dactylis* paved the way for the philosophy: *do not perform an artificial threshing of the seed sample by cutting off awns, straw, wings or other appendages, but report what you see in the sample*.

This idea was extended to other species during the following years: Appendages described in PSD 15 (pedicel on Apiaceae), PSD 38 (awns on *Oryza*), PSD 46 (stalk and leaves on clusters of *Beta*), PSD 62 (awn or rachis segment on *Hordeum*), PSD 47 and 51 (winged tree seeds), should not be removed since 1995/1998, but the content of seeds with appendages must be reported if representing 1% or more of the sample (Jensen 1995, 1998a).

In order to save time during the analyses and in agreement with the seed trade, the specification of MSU, inert matter and attached appendages are only reported on the certificate since 2003 if requested by the applicant.

The simplification of the purity analysis of species with high contents of multiple seed units (MSU) and the modification of the Pure Seed Definitions (PSD) mentioned above has saved a considerable amount of time for the laboratories testing those species.

5.9 Blowing of grasses

5.9.1 Blowing of Poa and Dactylis

Leggatt from Ottawa, Canada developed a new blower and introduced the climax blowing point for testing of grasses (Leggatt 1937, 1938, 1941a, 1941b). Leggatt and Porter from Iowa Experimental Station, USA, described the development of blowing, and a new concept of pure seed was introduced to seed technology (Porter and Leggatt 1942). In conclusion, the authors recommended AOSA and ISTA that serious consideration should be given to this new concept of pure seed.

Experiments with blowing of grasses continued at Iowa and the Iowa Air Blast Seed Separator for blowing of small seeded grasses was described by Åberg *et al.* (1945). The blowing activities at Ottawa lead to construction of the Ottawa Seed Blower. An example of the distribution of this blower is that it was bought by the Danish State Seed Testing Station in 1948 together with a calibration sample for 208 Canadian Dollars. The blower was used for research purposes during the following years, and blowing of *Poa* and *Dactylis* was compared with analyses using the hand method.

Leggatt (1950) presented a paper on "The use of a controlled-pressure blower in testing grass seed" at the ISTA Congress in 1950. The work with blowing continued under the leadership of professor Everson, Iowa, specific with comparison of methods and blowers (Everson 1958) and on comparison of the 'hand' and the 'climax' method for the purity analysis of *Poa pratensis* (Everson and Chen 1960). A number of experiments and comparisons between the hand method and the blowing method at a number of ISTA laboratories showed that both the time required and the variation between tests results were much reduced by blowing (Everson *et al*., 1962, 1965; Everson 1968b).

Based on those experiments, blowing of *Poa pratensis* was introduced into the ISTA Rules in 1966. Due to very small seeds, the variety Merion should, however, still be analysed by the hand method. The reason was that when samples of Merion were blown on a standard calibrated blower, a rather high amount of light seeds with caryopsis were blown into the light fraction and considered inert matter. The exception for the Merion variety was deleted from the ISTA Rules in 1985.

An increasing number of *Poa pratensis* varieties with small seed appeared on the market in the 1960'es and 1970'es (Schmidt 1975). When such small seeded varieties were blown by the standard method, the percentage of pure seeds was underestimated due to presence of seeds able to germinate in the light (inert matter) fraction. The Purity Committee studied this problem from the late 1990s, and in 2004 it was decided that varieties of *Poa pratensis* with a 1,000 seed weight <0.35g and included in table 3.5.2.A.5, should be blown with the usual blower setting for *Poa pratensis* multiplied by 0.82 (Jones and Kahlert 2005).

Blowing of *Poa trivialis* was included in the ISTA Rules in 1992 after comparative experiments with the hand method and as a harmonisation with the AOSA Rules (Jensen and Bülow-Olsen 1991, 1992). Laboratories in possession of a General Seed Blower should multiply the blower setting for *Poa pratensis* with the factor 0.82. Laboratories using other brands of blowers were asked to contact the ISTA Secretariat for further instruction.

A comparison of the uniformity of test results obtained by blowing and by the hand method indicated that blowing of *Dactylis glomerata* could be included in the ISTA Rules (Everson 1972, Everson and Hotchkiss 1977). The results showed that blowing of *Dactylis glomerata* gave less variation among seed laboratories compared to the hand method. The blowing method for *Dactylis* was included in the ISTA Rules of 1976.

5.9.2 Calibration samples of Poa and Dactylis

Calibration samples of *Poa and Dactylis* were essential for setting the blowers (Everson 1985). Calibration samples of *Poa pratensis* were produced at the seed testing laboratories at Iowa and Beltsville, USA in 1966. In 1990 Centre for Plant Breeding and Reproduction Research, the Netherlands produced a stock of 70 calibration samples. A new stock was produced in 2001 by the Danish Plant Directorate.

The calibration samples are composed of a mixture of the most commonly grown varieties, but is also attempted that a new stock of calibration samples produce results which do not deviate from the previous stock. The important calibration of seed blowers was demonstrated by e.g. Boeke at the purity workshop in Wageningen 1978 and at the workshop in Budapest in 1997 (Jensen 2000).

Artificial calibration samples were thought to be cheaper and able to secure a more uniform calibration of the seed blowers. The testing of various materials suited for such samples was for a number of years on the programme for the Purity Committee (e.g. 1989-1992), but so far it has not been possible to find an artificial material, which was superior to the calibration samples produced by blending of seeds from most commonly used varieties of *Poa pratensis* or *Dactylis glomerata.*

5.9.3 Blowing of Chloris gayana

The blowing of *Chloris gayana* was studied by the working group on tropical crops (Vennel 1978), and based on their recommendation blowing of *Chloris gayana* was included in the ISTA Rules in 1977. The Victoria Seed Testing Station, Australia prepared 15 calibrations samples of *Chloris gayana,* which were sold by the ISTA Secretariat.

The blowing method was removed from the Rules in 1986, mainly due to the difficulties in the supply of ISTA calibration samples.

5.9.4 Blowing of other grasses

As purity testing of several tropical grasses was very time consuming, attempts have been made to test some of these grasses according to the standard blowing procedure. Felfoldi (1972) developed a blowing procedure for *Paspalum dilatatum* and Easton (1972) compared blowing of *Paspalum dilatatum* to the hand method. Due to the great differences obtained under different temperatures and relative humidity of the air, it was not possible to find a well defined optimum calibration point. It was, accordingly, recommended to use a blower pressure 0.20 above the actual calibration point and check the components for misplaced seed.

In a similar study on *Chloris gayana,* Easton (1975b) found that blowing at a point where no empty seeds were left in the heavy fraction gave more reliable results than the hand method.

Blowing has also been used as an aid in purity testing of temperate grasses. The blowing point, for which no empty seeds were left in the heavy fraction after blowing the working sample for 3 minutes, was found for the grasses *Lolium perenne, Lolium multiflorum, Festuca pratensis, Festuca rubra, Festuca ovina, Poa nemoralis, Agrostis* sp. and *Cynosurus cristatus.* Blowing before the purity separation saved time, as only the light fraction needed to be inspected under reflected light for empty or undeveloped seeds (Jensen 1979e, 2000).

5.9.5 Development of blowers

The introduction of blowing of grasses in Ottawa was associated with the development of the Ottawa Seed Blower and the Iowa Air Blast Seed Separator. Other types were the South Dakota Seed Blower, Burrows Model, the Hearson Blower, the General Seed Blower and blowers produced in England, the Netherlands and Sweden.

The General Seed Blower became part of the standard equipment in many seed laboratories. In the ISTA Rules multiplication of the blower setting of *Poa pratensis* by the factor 0.82 is prescribed for purity test of *Poa trivialis* and for small seeded varieties of *Poa pratensis*; this factor applies for General Seed Blowers only.

5.10 Testing chemically treated seeds

Chemical treatment of the grasses *Poa* and *Dactylis* is requested from time to time. If the chemical treatment affects the blowing characteristics of the seed, the purity test of the sample must be performed by the hand method. Since 1995 the ISTA Rules have specified that the purity test and pure seed for germination of the species, tested by the standard blowing method, shall not be blown, but analysed by the hand method, and a sentence printed on the certificate, for example: *Because of the chemical treatment, the pure seed used for the germination was obtained by the hand method.*

6. Workshops and other training activities

6.1 Workshops

ISTA Purity Workshops were held at:

- Cambridge, United Kingdom 1967 (Slettenhaar 1968)
- Lyngby, Denmark 1973 (Anonymous 1974)
- Wageningen, the Netherlands 1978 (Anonymous 1979)
- Cambridge, United Kingdom 1985 (Tonkin 1985)
- Angers, France 1994 (Jensen 1995)
- Budapest, Hungary 1997 (Ashton 1997, Jensen 2000).

The ISTA Workshops have been very important for the development and implementation of the Rules for purity analysis and determination of other seeds by number as well as for the identification of problems and the preparation of new proposals for Rule changes.

The Workshops were often used to try new test methods such as classification of multiple seed units (MSU) before change of the Rules were presented at the ISTA Congresses. This doubtless facilitated the understanding of the Rules proposals among the ISTA membership.

The Workshops were also used to present the ongoing working programme of the Purity Committee, and as a means to inform ISTA members about the changes planned in the ISTA Rules. Through the discussions and exercises the participants became owners of the ongoing changes, and the Purity Committee got valuable feedback, which helped in the formulation of Rules proposals.

The summary of the workshop report from Wageningen 1978 (Anonymous 1979) clearly express the purpose of the ISTA Workshops from that period: *The primary object of the workshop was uniform interpretation of the International Rules for Seed Testing…The new Rules have been in force since 1976 and the analysts were therefore well prepared to discuss the needs for further elucidations of difficult or unclear paragraphs. The secondary object was to promote discussion of new ideas for testing seeds and of further amendments to the present Rules to be presented at the Vienna Congress in 1980.*

The use of special equipment in seed testing, such as blowers, sieves, magnetic separators, indented cylinders, image analyses and other kind of equipment, have also been part of most of the ISTA purity Workshops.

Information on seed collections, literature on seed morphology and not least the identification of selected crop and weed seeds have always been part of the programme (Jensen 1979a, 1979b, 1979c, 1979d).

Many regional workshops have been arranged, and they have played an important role in teaching the use of the ISTA Rules and in the understanding of the work performed by ISTA. Some were held in other languages than English. Reports on such workshops can be found in the ISTA News Bulletin and in Seed Testing International.

One example: a regional workshop organised by Jack Butler, Australia, was held in Colombia in 1984 on selected tropical grasses and legumes. It was concluded that the Pure Seed Definitions (PSD) for many of the species tested needed revision (Landenmark 1987).

The ISTA Workshops were also excellent opportunities to exchange information on analyses etc, and to meet colleagues and friends from other countries. The social events have always been important in the ISTA family.

6.2. Other training activities

Study visits, exchange of scientific staff and analyst have played an important role in improving the work and staff qualifications at ISTA seed testing laboratories. Participants have obtained general knowledge on how to perform the purity analysis and determination of other seeds by number as well as analyses of specific species. General purity courses have been shared between the Scandinavian countries and between Germany and Hungary.

A presentation of the training system for the seed analysts at the Wageningen Seed Testing Station was published by Schoorel (1962), and training seed analysts has always been of concern of the Purity Committee (Everson 1968a).

In the report of the Executive Committee of 1950-1953 (Anonymous 1953) it was mentioned that an agreement was signed between FAO and ISTA on training in seed technology for persons from developing countries. This lead to development of various seed programmes, which included workshops and training of staff at new and existing seed testing laboratories. The seed testing laboratories in e.g. Denmark, France, Germany, the Netherlands, Norway and United Kingdom have held training courses for seed analysts from developing countries. The courses were often paid by governmental or other national and international funds. An updated memorandum of understanding on transfer of technology and seed quality assurance was signed between ISTA and FAO in 2006.

7. Equipment

7.1 Development of facilities for purity analysis

It has always been of considerable importance to seed analysts to develop facilities suited for seed testing, and exchange of experience on various kinds of equipment have been on the agenda for Congresses, Workshops and study visits.

Several titles from the ISTA publications and products catalogue (2005) contain information of interest in relation to purity analysis and determination of other seeds by number. Of specific interest are the reports: Project seed laboratory 2000-5000 (Boeke *et al.* (1969), van der Burg *et al.* (1983), van der Burg (1994)); Survey of equipment and supplies for seed testing (Hardin 1973); Handbook for home made equipment (Madsen 1982).

The ISTA handbook on cleaning of agricultural and horticultural seed on small scale machines contains a description of the use of laboratory cleaning machines as an aid in testing for purity and number determination (Madsen and Langkilde 1987, 1988).

The early use of computers for registration of samples and requested analyses, calculation of purity results, check of tolerances and reporting on ISTA certificates, was presented at the ISTA Congress in Ottawa 1983 (Lave and Jensen 1983). Computers became later an important tool in the administration of most of the seed testing laboratories.

7.1.1 Balances

Weighing is important in seed testing, and the seed laboratories have, naturally, followed the development of suitable balances from mechanical types to the electronic models. Franck (1928), for example, described and evaluated a quick weighing balance with air damping and a chainomatic balance from USA.

Electronic balances, sometimes connected with computer systems, are now commonly used in ISTA seed testing laboratories.

Careful checking of the balances has always been an important element in the ISTA quality assurance programme in purity laboratories (see 12).

7.1.2 Optical aid

Identification of small seeds and inert materials requires optical aids. All seed laboratories use hand lenses and magnifying glasses. Use of small magnification glasses may be hard

on the neck and shoulders. Therefore, elevation plates were used at early stages, for example in Norway from 1930 and elevated working tables was introduced in Denmark around 1970 and in Norway from 1984 (Anonymous 1975, Ellingsberg *et al.,* 1984). The diaphanoscope, described under 7.1.5, improved also the working condition for the analysts.

A working group on optical aid was active in the period 1968-1971 and produced a report on new optical aids in routine analyses of purity seeds (Boeke 1969).

7.1.3 Microscopes

When binocular microscopes occurred on the market, it became possible to adjust the microscopes according to the need of the seed analysts. This also improved the recognition of specific seed characteristics as the details on the seeds surface were easier to see, and it was possible to adjust the magnification according to the size of the seeds.

Identification of certain types of seeds relies on specific microscope techniques. *Orobanche* seeds, for instance, are identified using an incident-light fluorescence microscope. The lignified testa cell walls render the seeds fluorescent, thus enabling visualisation of the fine wall ornamentation typical of each species (Joel 1987).

Scanning Electron Micrographs (SEM) has often been used for examination of seed morphology of closely related species. Many papers on this topic, marked SEM, are listed in Bibliography on Seed Morphology (Jensen 1998b).

7.1.4 Inspection stations

An inspection station for determination of other seeds by number was constructed in Oregon, USA (Hardin 1974). During examination, the working sample was transported under a microscope by vibrators and the weed and crop seeds looked for could be removed by a vacuum needle. A modified Oregon Microscopic Station with only one vibrator was constructed and used for examination of *Poa* spp., *Phleum pratense* and *Trifolium* spp. for content of *Rumex, Avena fatua* and *Cuscuta*. Use of this microscopic station saved a considerable amount of time compared to the hand method (Jensen 1979e).

7.1.5 Diaphanoscope

Transmitted light has been an important tool especially for the examination of grasses for the presence of caryopses. Various models have been used (Frisak 1936), and the transmitted light is frequently part of the working tables or built-in the microscopes (van der Burg 1994). During Workshops in 1973 and 1978, the difficulties in observing the size caryopses in grasses were frequently mentioned by the participants. The reply from Elisabeth Felfoldi, Australia was: *use a diaphanoscope in a dark room.* Not many analysts, however, liked the idea of being placed in a dark room for hours, analysing grass samples for caryopses.

7.1.6 Sieves

The use of sieves in purity analysis of *Beta* was included in the ISTA Rules already from 1931, as only clusters retained on a 2mm sieve were considered pure seed, whereas the clusters passing this sieve were classified inert matter.

Before an effective size grading was introduced in cleaning of Beta, it was due to different sizes of the clusters, difficult to obtain a representative sample for the germination analyses. Aiming to overcome this problem, the Rules of 1938 prescribed that from a sample of 50g all extraneous matter should be removed and the remaining part of the sample divided into 5 fractions by a set of sieves with 5, 4, 3 and 2.5mm slits. The clusters retained in each sieve should be counted, and the number of clusters to be taken from each sieve should be calculated, aiming to obtaining the 100 clusters necessary for each replicate in the germination test.

The Beet Seed Committee tried various methods for drawing pure seed for germination, but was not able at this stage to recommend a method, which could replace the time-consuming sieving and counting method (Stahl 1950a).

Improved cleaning techniques reduced the problem, and since 1966 pure seed for germination have been obtained from the pure seed fraction. The use of the 2mm sieves in purity analysis of *Beta* was removed from the ISTA Rules in 1995 to harmonise with the AOSA Rules

The use of sieves as an aid in purity and number count analyses has always been a standard procedure at most seed testing laboratories. In Norway, for example, a sieve is shown on a photo of the purity laboratory taken around 1930 (Ellingsberg *et al.,* 1984).

7.2 Size grading

After introduction of monogerm *Beta* seeds, which were planted by precise drilling machines, a uniform size of the seeds and the seed pellets became important. Therefore, seed grading became part of the ISTA Rules of 1976 (Appendix A).

In the Rules it is stated that "The 'Bonn' Screening apparatus…with the requisite round-hole screens and automatic switch-gear for regular interruption of the reciprocations may be used."

This is one of the few cases where a specific brand is mentioned in the ISTA Rules. The reason is, most likely, that is was a problem with different brands of screening apparatus to obtain uniform results between laboratories.

A uniform and precise size of the holes in the sieves, used for the size grading, is also very important for reproducing the results. A method to test the reliability of the sieves, used for size grading, was developed by Kruse and Steiner (1994).

7.3 Image analyses (seed scanner)

Westerlind (1988) described the development of a seed scanner, a computer–based device for determination of other seeds by number in cereal seeds. The seed scanner sorted the seed sample into two fractions. One fraction contained typical seeds of the species examined and the other deviating seeds and various impurities. Only the latter fraction, usually representing only 0-15 pct. of the sample, needed to be examined by the analyst.

The seed scanner combined with manual examination could be calibrated to work with at least the same accuracy as samples tested by manual analyses. A considerable amount of time could be saved when the seed scanner was used on cereals. The system has been used since 1988 at five Swedish seed testing laboratories and later in Norway, Denmark and in a few other countries.

A working group on image analyses was formed in 1989 with Ulf Kjellström, Sweden, as leader. The equipment was presented at the ISTA Congresses in 1986 and 1995 and during the purity Workshops in France in 1994 and Budapest in 1997 (Oliveras 2000). A Danish-developed seed scanner was at the workshop in Budapest presented by Skjödt (2000).

Travis and Draper (1985) described a computer based system for recognition of the size and shape of seeds from 49 crop- and weed species. Automatic identification by colour machine vision provided improved identification of four weed seeds, compared to black and white images (Petersen and Krutz 1992). Chtioui *et al.* (1996) used colour imaging combined with discriminate analyses and artificial neural network for seed identification, and Gupta *et al*. (2005) introduced the use of a computerised database for seed identification. McDonald *et al.* (2001) demonstrated the potential use of a flat bed scanner for making seed images of seeds and fruits.

The development of computer imaging and sorting of seeds will without doubt continue. By avoiding cutting off inert material in multiple seed units of grasses and deleting cutting off wings and appendages on seeds (see 5.8) the ISTA rules for purity analysis are prepared for this development.

7.4 Cleaning equipment

Cuscuta spp., being a troublesome weed in warmer countries, has always been a serious seed testing problem. A Dodder Seed Committee was formed at the seed Conference in Copenhagen in 1921, which reported on the activities at the Cambridge Congress in 1924 (Degen 1925). Lengyel (1938) informed at the Congress in 1937 that construction of an electromagnetic cleaning machine, able to remove *Cuscuta* seeds from seed lots, had reduced the dodder seed problem considerably. The magnetic seed cleaning machine (a dodder mill) can be used when samples of *Trifolium* and *Medicago* are examined for content of *Cuscuta*, and an indented cylinder has been used for testing *Poa, Festuca* and *Dactylis* for content of *Cuscuta* (Jensen 1979e, Jensen *et al.,* 1987).

Landenmark (1978) found that mechanical separation of wild oat (*Avena fatua*) seeds from cereal samples could be made by use of Bardex Q 1. The apparatus was equipped with an inclined moving conveyer belt with a suitable surface structure. The Bardex cleaner was able to separate wild oats from samples of wheat and 2-row barley. For other kinds of cereals the equipment was less efficient.

Other laboratory cleaning equipment (sieve and air cleaner and indented cylinder) for removal of individual impurities (e.g. stones) having an undue effect on purity results (ISTA Rules 3.5.2.A.7) or for concentrating the weed seed sought for in a smaller fraction have been used as an aid in the purity analysis (Madsen and Langkilde1987-1988). The calculation of purity analysis including cleaning is explained by Jensen (2000).

8. Seed identification

Since the dawn of seed testing, correct seed identification has been the basis for purity analysis, determination of other seeds by number and for provenance determination (see

chapter 13). Many of the early published papers included information for all three types of analyses (e.g. Grisch 1935).

Seed collections, seed illustrations and descriptions of seed morphology have been valuable tools in the identification of unknown seeds. In 1977 a working group on identification of seeds was founded, which collected information on seed collections as well as on seed morphology (Jensen 1979a, 1979b, 1998b).

8.1 Seed collections

Seed collections, covering the crop and weed seeds tested in the laboratory as well as those occurring in the proficiency test samples submitted by ISTA, are very important tools for the seed identification.

Many botanical gardens have supplied seeds to the seeds testing laboratories. Beautiful seed collections could be obtained from Hungary in the 1930'es, and seed collections have been offered for sale from the ISTA seed laboratory at Hohenheim, Germany and from Angers, France since around year 2000.

The experience in how to establish and maintain seed collections was presented at ISTA Workshops in Wageningen 1978 (Jensen 1979d) and Budapest (Jensen 2000). The presentations covered different kind of seed collections, arrangements of seeds in collections, types of containers, control of insects and use and maintenance of seed collections.

Preparing a universal list of weed species has been on the working programme for the Purity Committee since 2004, and the supply of such seeds and training in identification of weed and crop seeds are still important elements in the working programme for the Purity Committee in 2006.

8.2 Illustrations and descriptions of seed morphology

The Working Group on Seed Identification also collected information on handbooks and articles with illustrations and descriptions of seeds and fruits from all over the world. This information was published in a bibliography containing 3,775 references. The papers, all printed before 1990, contained descriptions of seed morphology of 3,900 genera (Jensen 1998b). The morphology of the seeds was described by use of keys and illustrated by drawings, photos and pictures taken by electron scanning microscopes.

Gunn and Seldin (1977) prepared seed and fruit characters for a computer identification and discussed five useful computer programmes.

At the Budapest workshop, specific training was given in the use of botanical characters for identification of seeds (Leist 2000a), identification of seeds of *Lathyrus* and *Vicia* (Leist 2000b), *Avena* species and spontaneous crossings between them (Leist 1981, 2000c), of the Brassicaceae family (Andersen and Skjödt 2000) and of *Rumex* and *Silene* seeds (Andersen and Jensen 2000).

Bernard (1998) described the comparative seed morphology of five *Lolium* species growing in France, Marin *et al.* (1998) examined the seed sculpturing of selected *Vicia* species and Allison *et al.* (2001) the florets of *Bromus riparius* and the closely related *Bromus inermis.* In these papers keys, drawings and scanning electron pictures were used when describing differences between species.

A guide with colour photos of some 200 species of agricultural, horticultural and weed species has been published by NIAB, United Kingdom (Jones *et al.,* 2004), and a descriptive and illustrated seed-book on 175 weeds was worked out by GEVES France. A French edition was published in 1999 and an English edition in 2004 (Dragos 2004).

More than 1,400 images of seeds and fruits have been made available online by professor, Dr. Arnold Larsen, Colorado State University, USA (www.seedimages.com). The web site includes colour images of seeds as well as descriptions and keys to the described species.

Dr. John H. Wiersema and his colleagues in GRIN Taxonomy, United States Department of Agriculture/Agricultural Research Service have added over 3,800 seed/ fruit or embryo images or drawings to GRIN, with plans to add another 5,000 in the future. They can be retrieved by family, genus, or species from the web page (http://www.ars-grin.gov/cgi-bin/npgs/html/taxassoc.pl) and are also linked from the relevant family, genus, or species pages in GRIN. Many of the same images are also available at the "Family Guide for Fruits and Seeds" on Systematic Botany and Mycology website which also provides detailed seed and fruit descriptions for most vascular plant families.

Professor Miller McDonald and his staff at Ohio State University, USA, have on the website (www.oardc.ohio-state.edu/seedid) placed a number of images of cultivated and weed species. The web site includes a very useful basic and advanced seed identification quiz.

8.3 Chemical methods

Chemical methods have been utilised as an aid for purity analyses mainly for distinguishing between species, for which visual identification at the species level is either not possible or uncertain.

The fluorescence test for separating *Lolium perenne* from *Lolium multiflorum* has been widely used as a supplement to the purity analysis (ISTA Rules 8.9.4). The method was described by Gentner (1929b), and improvements of the technique have been published in various papers, among others Nieser (1953) and Harrison (1954).

A number of papers reported that the non-fluorescent *Lolium perenne* did include some seeds with fluorescent roots, and *Lolium multiflorum* included some non-fluorescent seedlings. Plants that deviated in fluorescence all had the morphological characteristic of the species, and seedling root fluorescence was not linked to species and botanical characteristics (Dorph-Petersen 1934; Jensen and Langkilde 1965, Jensen 1980, Okora *et al.,* 1999). The content of seedlings, deviating in the fluorescent character, varied from 0 to 20%.

Considerable differences occurred both between samples and cultivars (Baekgaard 1955; 1962; Jensen and Olsen 1975; Jensen and Olesen 1975). Due to the variation in the content of fluorescent seeds, a content of 5% fluorescence in seed lots of *Lolium perenne* was allowed in the USA (Justice 1950). To day, the method is rarely used for separation between Lolium species, but some countries still used it in description of new varieties.

Elekes (1975) used the differences in fluorescence and chemical colour reaction to separate between caryopses of *Poa pratensis* and *Poa trivialis* and between *Phleum pratense, P. nodosum* and *P. bertolonii.*

Seeds of *Cuscuta* spp. and *Orobanche elatior* turn yellow after treatment with a 0.1 N solution of soda-lye whereas no colour reaction was found in a similar test of more than 100 other weed species (Grosbüsch 1948).

The phenol reaction (ISTA Rules 8.8.1) was used for separating seeds of *Vicia lutea* and *Vicia striata* (Germ 1937) and for identification of the Merion variety of *Poa pratensis* (see 5.9.1). Schuphan (1948) and Papp (1973) used a spectral photometric method for identification of species and varieties of the genus *Brassica.*

Vieritz (1992) used gel electrophoresis as an aid when distinguishing seeds of four species of *Sida*.

Surveys on the rapid chemical identification techniques used in the ISTA laboratories have been published by van der Burg and van Zwol (1991) and in the ISTA Variety Testing Handbook (ed. Payne 1993).

References to other papers, describing the use of chemical methods in seed identification, can be found in Jensen (1998b).

The fluorescence method for separating *Lolium perenne* and *L. multiflorum* has, as indicated above, been commonly used. Other chemical analysis has only been rarely used, as the number of seeds available in most cases was limited and the methods described frequently required specific equipment and chemicals.

9. Relation to other ISTA Committees

9.1 Bulking and Sampling Committee

9.1.1 Dividers

Representative and correct working samples have been the responsibility of the Bulking and Sampling Committee since the creation of this committee. The subject has been an important item for the Purity Committee as well, because the accuracy of both the purity analysis and determination of other seeds by number are strongly influenced by the dividing method.

Thomson and Doyle (1955) compared the halving and the random cups methods for *Lolium perenne, L. multiflorum, Trifolium pratense, T. repens, Brassica* spp. and *Lactuca sativa.* The variation between duplicate samples was not significantly different for the two dividing methods.

Madsen and Olesen (1962) compared working samples obtained by means of a spoon and by a Pascall divider. The variation in the purity results after sampling by the divider was far greater than the corresponding variations after sampling by the spoon method. The experimental work with this divider was discontinued, as comprehensive unpublished experiments with the Boener divider demonstrated that this divider gave more reliable results. Accordingly, since 1969 the Boener divider replaced the hand method for drawing working samples at the Danish State Seed Testing Station.

Warwick (1978) compared five methods: random cups, centrifugal divider and three types of soil divider with varying number and widths of channels for obtaining working samples from submitted samples. The optimum results of the dividing depended on the size of the seeds used.

These published and unpublished experiments are only examples on the extensive accumulation of experience, which lead to that in the ISTA Rules (2.5.2.2) of 2006 in total eight types of dividers/dividing methods have been approved for drawing working samples for purity tests and number determinations. Recently, methods for checking whether dividers divide equally and without selection have been an important issue in the ISTA quality assurance programme in the purity laboratories (Kruse 2004).

9.1.2 Size of working samples

Table 2A in the Rules, which are under the responsibilities of the Bulking and Sampling Committee, contains the mass (weights) of the working samples for purity tests and for count of other species (described in 5.2). As the size of the working samples influences the accuracy of the results as well as the work involved, the Purity Committee has been involved in defining the size of the working samples as well as providing seed mass (weights) for new species introduced in the Rules.

9.2 Coated Seed Committee

The introduction of coated seeds, seed pellets and seeds in bands created a number of new problems in purity tests and determination of other seeds by number. The Coated (Pelleted) Seed Committee was formed in 1968, as the ISTA Rules for these tests at that time were not suited for this kind of seed structures. This committee proposed a new and specific set of Rules for testing seeds covered with pelleting materials.

Test methods for pelleted seeds were included as Appendix A in the ISTA Rules of 1976 and as a separate chapter in the ISTA Rules of 1985. It was then decided that the committee should be discontinued and any work considered necessary and related to Purity Committee should then be taken on by this committee (Tonkin 1987).

Gáspar (1972) reported on the Hungarian experience in testing pelleted beet seeds, and testing various types of coated seeds were part of the ISTA Workshop in Denmark in 1973 (Ader 1974).

9.3 Flower Seed Committee

Only few ISTA laboratories test a wide range of flower seeds. Comprehensive contributions to the development of purity test of flower seed species have been published by the private Ransom Seed Laboratory, California, USA (Atwater 1972, 1980). The Flower Seed Committee was formed in 1971, and has contributed to the development of the ISTA Rules (Barthodeiszky 1975, 1978, 1981; Jensen 1984b, 1987; Timmann 1989, 1992, 1995; Mazor 1998, 2001).

The cooperation between the Flower Seed Committee and the Purity Committee has been facilitated by at least one person being member of both committees, and most of the pure seed definitions (PSD) for flower seeds were developed together with the Purity Committee working group for pure seed definitions.

9.4 Forest Tree and Scrub Seed Committee

The pure seed definitions for forest tree seeds were developed in cooperation between the Forest Tree and Scrub Seeds Committee and the Purity Committee working group for pure

seed definitions (PSD) including drawings (van der Burg 1991). The coordination between the Purity Committee and the Forest Tree and Scrub Seeds Committee was secured as at least one person from the latter committee was member of the Purity Committee through most of the period.

9.5 Nomenclature Committee

Internationally accepted plant names are necessary for a correct and uniform reporting of the species found in the tests. Mentz (1937) and Davidson (1950) recommended that ISTA should follow the names accepted by The International Botanical Congress on Nomenclature.

ISTA Congress in 1950 accepted a resolution on Uniform Nomenclature in which it was pointed out that the change of species names of plants of economic importance created confusion in the field of seed testing.

Davidson (1953) and Nilsson-Leissner (1962), for instance, reported as early activities in the Nomenclature Committee of ISTA on the contact with The International Botanical Congress. ISTA accepted the International Code of Nomenclature, and the ISTA Nomenclature Committee has since provided valuable information on plant names. The plant names in the ISTA Rules as well as in the ISTA List of Stabilized Plant Names (1st edition 1966)) were important tools in assuring a uniform use of plant names on ISTA certificates and in ISTA publications.

Recently, the Stabilized Plant Names is available on the internet through the GRIN database (this is easily accessible via a GRIN link from the ISTA web page for the Nomenclature Committee). Names on the ISTA List of Stabilized Plant are stabilized for a period of six years. Accordingly, major revisions of the list are performed every six years (Wiersema 2005). The stabilized plant names and GRIN are widely used also within the EU (Jensen *et al.,* 2003, Arnklit *et al.,* 2007).

9.6 Rules Committee

It has always been of outmost importance for the quality of the Purity Rules Proposals that the content and the phrasing were in harmonisation with other parts of the ISTA Rules. Therefore, all proposals were reviewed by members of the Rules Committee since the foundation of Purity Committee. This has improved both the clarity and the wording of the proposals before presenting those to all ISTA members prior to the Congresses and also during the discussions at the Congresses.

10. Calculation and reporting of results

Adoption of international seed certificates was discussed both at the Congress in Copenhagen in 1921 (Bruijning 1922) and at the Cambridge Congress in 1924 (Anderson 1925).The international certificate was finally adopted at the ISTA Congress in 1931. The issue of ISTA certificates was a continued concern (Stahl 1950b), and in 1956 an International Certificate Committee was formed. The report from this committee included discussions of repeat of tests, reported on ISTA Certificates (Stahl 1960).

In the first ISTA Rules of 1931 it was mentioned that for samples of "any kind of seed not listed, use a quantity approximating to that given for a similar sized seed". Since 1976 the issue of ISTA certificates has been restricted to the species mentioned in the ISTA Rules.

The first ISTA Rules also stated that the results of a purity analysis shall be given to one decimal place. When the percentage was 75% or less, the results should be given in whole figures. The reporting of low purity results in whole figures was removed in 1953.

During the ISTA Workshop in Cambridge in 1985 it was discovered that the rounding off for purity results was performed according to at least three different methods at the ISTA Laboratories. A working group on calculation of purity results was formed in 1986 and revised Rules for calculation and rounding off purity results was accepted during the ISTA Congress in 1989.

In 1995 it was accepted that when the mass (weight) of the working sample tested for purity deviated from that prescribed in table 2A, column 4, the actual mass (weight) examined shall be reported on the certificate.

Since 1931 the content of other seeds and inert matter and from 1984 the content of MSU should be reported on the ISTA Certificate if representing 1.0% or more of the sample. To save time during the purity analysis, since 2001 the occurrence of such structures should only be reported if requested by the sender of the sample or when specification is needed according to national or international seed regulations.

The determination of other seeds by number can be performed for one species (e. g. *Avena fatua*), a few selected species (e. g. *Avena fatua* and *Cuscuta* sp.) or for all other seeds. Those examinations can be performed in the weight specified in table 2A, column 5 (or in a weight corresponding to 25,000 seeds) or in a reduced amount of seeds. In order to ensure that the information on the certificate is always unambiguous, the following endorsements were introduced in the 1976 Rules:

- A *Complete test* is one in which the whole working sample is searched for all other seeds present
- A *Limited test* is one in which the search is restricted to designated species in the whole working sample
- A *Reduced test* is one in which only part of the working sample is examined.

In 1998 the following combination was added:

- *A Reduced-limited test* is one in which less than the prescribed weight of seed for a working sample is examined for stated species only.

11. Proficiency testing

Since the very beginning of ISTA uniformity in seed testing has been the goal. Prior to the Congress in Copenhagen in 1921, 25 different seed samples for purity and germination

tests were submitted to 22 laboratories (Dorph-Petersen 1922). The results disclosed considerable differences between the purity and germination results.

The activities on comparative testing continued, and the Referee Test Committee was formed in 1962. The name was changed to the Proficiency Test Committee in 2003, as this name suits the present international terminology.

The Purity Committee has cooperated with the Referee/Proficiency Test Committee through specific working groups responsible for elucidating the purity testing problems and for preparing samples for distribution. An example: the leader of the working group 1995-1998 Didier Demilly, France prepared a review of the problems related to preparing and analysing the results of referee tests on purity and determination of other seeds by number. The Proficiency (Referee) Committee agreed to distinguish between: seed retrieval, seed identification and reporting in the evaluation of the results, and this system were used in the Report of the Referee Committee 1995-1998 (Ednie 1998).

Proficiency testing, workshops, internal training, improvements of the ISTA Rules and quality assurance have all been essential steps on the way towards uniformity in purity and number count determinations.

12. Quality assurance and accreditation

The accuracy and repeatability of seed testing has been important for ISTA since the first ISTA Rules were published in 1931. They included defined sizes of the working samples, Rules for performing the analyses, tolerances between duplicate tests and guidance in reporting the results.

Also the equipment was of concern. The first ISTA Rules state: *The accuracy of the balances should be often checked (at least once a week) by the chief analyst.*

The continuous effort to improve both the content and the interpretation of the ISTA Rules secured that ISTA was well prepared when the international trend towards quality assurance appeared in the 1980'es.

A working group on quality assurance was formed in the Purity Committee in 1995. The aims of the work were:

- Examine/revise the ISTA Rules for purity and number count test in relation to quality assurance
- Describe methods which can be used for testing equipment used for purity and determination of other seeds by number.

The subjects were discussed during the ISTA Workshop in Budapest, 1997. Cooper (2000) informed about accreditation and quality assurance of ISTA laboratories and Demilly (2000) contributed application of accreditation and quality assurance in a purity laboratory. The requirements included: internal procedures, sample and record management, staff organisation and training, seed collections, equipment documentation, operation and control, control of degree of accuracy, handling of complaints, feed back and corrective actions.

A number of initiatives including accuracy, staff training, and calibration of equipment and reference materials are listed in the Purity Committee report of 1995-1998 (Jensen 1998a), and a poster on establishment and maintenance of seed collections in relation to quality assurance was presented at the ISTA Seed Symposium at Angers, France (Jensen 2001).

13. Provenance determination

At an early stage of seed testing, determination of provenance (origin) of the seed lots was an important analysis. As the analysis is closely related to purity and determination of other seeds by number, the development of the test is briefly summarised below.

Volkart (1922) reported that Stebler at the first European Seed Testing Conference in Hamburg in 1906 presented the possibilities for provenance determination in seed lots. Provenance was determined by the composition of weed and other crop seeds typical for specific productions areas. The background for the need of this test was the increased international trade, in which seeds of landraces, adapted to warm climates, sometimes were sold in cooler areas, where such plants did not survive the low winter temperatures. Spreading of noxious weeds like *Cuscuta* was also a problem.

Volkart (1922) presented examples of examinations of *Trifolium pratense* samples from Mid France, Czechoslovakia and Switzerland, where both the dominating species and species specific from the area were indicated. During the discussion it was recommended also to pay attention to other impurities like earth, small stones, shells etc.

During the ISTA Congress in Rome in 1928, Gentner (1929a) presented a contribution to a monograph on determination of the provenance of clover and grass seed.

The work continued and Gentner (1937) published a revised article on the provenance determination of clover seeds. The presentation covered both examinations for content of weed seeds as well as the use of minerals and other impurities as an aid in the provenance determination.

A valuable tool in the provenance determination was provided in *Proceedings of the International Seed Testing Association,* **9,** 413-420, where the names and drawings are shown of characteristic seeds and fruits, originating from eight locations in Europe and from North- and South America. These seeds and fruits were used as indicators of provenance, or in other words that the seed lot was likely to originate from one of the specific areas, if the seed lot contained one or more of the indicator species.

The interest in the test remained until 1970'es, and as late as in 1978 Berdejo Iznardi (1978) examined whether it was possible, by use of other crop seeds and weed seeds found in purity analysis from 1974-1976, to determine the provenance of three Spanish varieties of alfalfa (*Medicago sativa*). *Prunella vulgaris* was, for instance, detected as a possible indicator of provenance for the Aragon cultivar.

The test was used for a limited number of samples in the period 1960-1970, as an indicator of, whether a seed lot contained any other seed or other impurities, which could indicate that the seed lot was not grown in the area. If no such seed or impurities were found, a sentence was placed on the certificate, informing that the seed lot did not contain

any impurities, which could indicate that the seeds were not grown in the country. The provenance determination was very important in the first 60-75 years of seed testing, but the decreasing number of impurities due to improved seed processing made reliable provenance determination difficult. Combined with the increased use of the OECD seed certification the test lost its importance and was removed from the ISTA Rules in 1976.

14. **Conclusions**

Adoption of the first ISTA Rules and the international certificate in 1931 was the first very important steps after foundation of ISTA in 1924.

A number of substantial changes were introduced into the ISTA Rules for purity analysis and number count determinations from 1931 to 2006 : purity tests according to the quicker method (1950); introduction of the three component rule (1974); pure seed descriptions (1976); blowing of grass samples (1966, 1976, 1992); testing of grasses containing multiple seed units (1984); working samples for purity and determination of other seeds by number based on the mass (weights) corresponding to 2,500 seeds (1966) and 25,000 seeds (1990), respectively, and formulation of specific Rules for testing of coated and pelleted seeds (1976, 1985)

In the same period the ISTA Rules has also been enlarged from 41 species/genera in the in 1931 to 898 species/genera in 2006.

A consequence of this development is that it is now possible to adjust the testing method according to the species tested and to perform the analyses with improved accuracy and at the same time save time during the analyses.

The technical development of for example microscopes and availability of seed images on the internet have contributed to this positive development. The progress in development of computer imaging and sorting of seeds will without doubt continue. By avoiding cutting off inert material in multiple seed units of grasses and deleting cutting off wings and appendages on seeds, the ISTA rules for purity analysis are already prepared for this development.

The valuable progress obtained from 1924 to 2006 is based on contributions from many individuals and support from many countries, which have contributed and cooperated in the preparation and implementation of the ISTA Rules.

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