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Seed sampling -
Basic principles and procedures

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27th of April 2023

Objectives and Outline

- Basic understanding how seed sampling is performed in the seed sector
- Presentation of basic principles on seed sampling
  - Theory and practical work
    - To define the seed sampling intensity
    - Sampling equipment, preparation of the submitted sample for further analyses
    - Seed sampling techniques and disinfection of all the equipment to avoid cross contamination with infectious seeds

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Importance of seed production and seed quality (1)

- Seeds are as an agricultural input at the beginning of food and feed production
- Seed market was valued at $ 58,5 billions in 2020 and is expected to reach more than $ 100 billions in 2030 (https://www.alliedmarketresearch.com)
- Only 30 crops dominate and provide more than 50% of the world wide calory consumption
- Spectacular and questionable concentration processes in the seed sector, less concentration in EU market than in the US
- Maize and vegetable seed markets are highly dominated by few actors; mainly vegetable seeds are traded worldwide

Importance of seed production and seed quality (2)

- Breeding activities are focused on only few species worldwide (30)
- nowadays breeding progress is considered most important
  - to adapt to climatic changes
  - to increase the sustainability of the agricultural production
  - to allow changes in consumer behaviour (e.g. replacement of meat)

✓Investments in seed production enables the agri-food sector to secure increasing demand of food and feed in quantity and quality

✓Achievement of sustainability goals of the agricultural value chain

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Seed quality testing in Switzerland

- at 19th century farmers are buying seed on seed markets
- Fraudulent activities occurred (e.g. red stones in clover seeds)
- Demand for a neutral seed quality control, thus in Switzerland the seed testing laboratory started its activity in 1879
- Agricultural research stations began their breeding activities began at the 20th century
- First seed producing cooperative was founded in 1911; “swisssem” the branch organisation in 1921
- Field inspection started in 1913, ensuring varietal authenticity and purity
- Seed supply mainly for the domestic market (cereals, potatoes, red clover), grasses are multiplied abroad

International role of the ISTA

- Aim: Demand to harmonize methodology to obtain equal results
- ISTA was founded in 1924 to uniform seed quality evaluation worldwide with appropriate methodology
- Handbook of tolerances and measures of precision was published in 1963 by Miles (Purdue University, USA)
- internationally agreed rules for seed sampling and testing were actualized and published yearly
- Provide international seed analyses certificates (OIC) and training
- Carry out proficiency tests and laboratory accreditation
- assure the further development by 10 committees of experts

ISTA's mission on representative seed sampling

- General understanding: correct sampling of a lot is a prerequisite for a reliable quality estimation of a seed lot in the laboratory
- Sampling procedure itself does not influence the results
- Tasks of the experts of the bulking and sampling committee
  - yearly update the 2th chapter of the ISTA rules (download free of charge, www.seedtest.org)
  - ISTA Handbook of Seed Sampling (3rd edition, 2022) covers all principles from sampling to quality assurance of the sampler and its equipment

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ISTA's mission on representative seed sampling for seed health methodology

- Chapter 7: statistically validated seed health testing methods
- Important due to seed borne diseases
- Introduction of new diseases by importing infected seeds
- Seed borne diseases can influence seed germination and field establishment; infection can indicate a seed treatment
- Actually 30 methods are validated by proficiency testing and interlaboratory validation tests (free download)
- 7-028: Detection of infectious tobacco mosaic *virus in Solanum lycopersicum* => *minimum recommended sample size: 3'000 seeds*

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Responsibilities of the seed sampler (1)

- Fulfil its task in independence to the seed producing company, confident person with practical concern on seed quality aspects
- Carry out the seed sampling according to the procedure described in Chapter 2 and the quality assurance of the accredited laboratory
  - most National Designated Authorities for seed certification combines important aspects in their own seed sampling directive, adapted for national purpose within legal seed certification
- Appropriate use of sampling equipment
- Determine the necessary sampling intensity for the lot concerned
- Correctly use of the different sampling techniques

Responsibilities of the seed sampler (2)

- Respecting sanitary measurements to avoid cross-contamination
- Preparing the submitted sample for the laboratory
- Regular participation in training activities and measures of quality assurance (e.g. accompanied sampling and double sampling)
- Accept to present its knowledge at laboratory audits
- If conditions for sampling are not respected, sampling is refused (e.g. obvious visible heterogeneity)
- Seed sampler = accredited person of a ISTA seed quality laboratory

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Seed and phytosanitary sampling - common principles and differences (1)

- Seed sampler collects its samples in the warehouse
- Submitted sample always consists only seed from **one particular, well identified and described lot** (e.g. number of containers, weight, labelling), unique seed lot identification, seed origin is described, replacement of seed taken is possible (e.g. vegetables in small bags)
- Sampling according to ISTA rules (Chapter 2)
- Seed testing mainly with visual evaluation based on photographic specifications (e.g. pure seed determination or normal seedling development according ISTA rules)

Seed and phytosanitary sampling - common principles and differences (2)

- Phytosanitary inspector collects its samples during transport (e.g. border control at the airport) or in the production facilities (e.g. greenhouse), transport accompanied with or without phytosanitary certificate and plant passport (e.g. Europe)
- Sampling according ISPM 31, often **composite sample of several lots**, no seed replacement possible, infected plant tissue with considerable risk for cross-contamination of the equipment
- Plant material testing based with highly sensitive methodology (e.g. ELISA or PCR)

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General requirements for seed sampling

- Seed sampling protocol with address of the applicant
- Species and variety
- Lot reference number, kind of labelling and sealing
- Size of the lot
- Number and size of the containers
- Type of containers
- Additional chemical treatment or coating of the seed
- Date and place of sampling & authorization number and signature

✓Complete information allows traceability

Heterogeneity of seed lots - a problem

- Different growing conditions of the plants in the field (e.g. water, light, soil)
- Different harvesting conditions (e.g. seed moisture)
- Seed processing aims the elimination of impurities and seed size harmonisation to some extent
- Seed segregation is still occurring during seed transport and bin filling
- During storage, seed vigour and germination are likely to change

✓thus perfect uniformity doesn't exist

✓Reduction of variability by limiting maximal seed lot sizes and correct sampling intensity according to Chapter 2

Maximum lot size & sampling intensity

- Maximum seed lot sizes vary from 40 t for Maize, 30 t for cereal crops and 10 t for herbage species and vegetable seeds
  => seed lots of smaller seeds show greater variability
- To take an appropriate number of primary samples is necessary to obtain a representative sample
- Positioning of sampling may be selected by random or by a systematic plan (e.g. every tenth bag or every two minutes in the seed stream)
- As principle the same number of primary samples of each container must be taken to avoid under- or over representing in the submitted sample for analyses
- With increasing the lot size the results' heterogeneity increases also

Sampling intensity - crucial for representativity

- Sampling intensity is based on statistical requirements
- 3 different situations according to the weight of the container
  - Weight of the container smaller to 15 kg
  - Weight of the container between 15 to 100 kg
  - Weight of the container larger than 100 kg (e.g. big bags and automatic sampling in the seed stream)
  - Primary sample is obtained from one single sampling action
  - If the number of containers is below 15, then each container ought to be sampled

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Sampling intensity and minimum submitted sample for analyses (1)

- Containers' size between 15 and 100 kg

Number of containers in the seed lot	Minimum number of primary samples to be taken
1 to 4	3 primary samples from each container
5 to 8	2 primary samples from each container
9 to 15	1 primary sample from each container
16 to 30	15 primary samples, one each from different containers
32 to 59	20 primary samples, one each from different containers
60 and more	30 primary samples, one each from different containers

Source: *Table 2A, ISTA rules 2023, p. 2-3*

- Example lot of perennial ryegrass: 20 bags with 15 kg each

⇒ at least 15 primary samples

⇒ 60 g as submitted (at least 25'000 seeds)

Sampling intensity and minimum submitted sample for analyses (2)

- Containers' size more than 100 kg

Lot size	Minimum number of primary samples to be taken
Up to 500 kg	At least 5 primary samples
501 to 3'000 kg	1 primary sample for each 300 kg, but not fewer than 5
3'001 to 20'000 kg	1 primary sample for each 500 kg, but not fewer than 10
> 20'001	1 primary sample for each 700 kg, but not fewer than 40

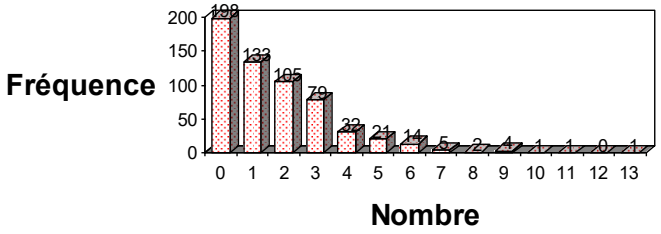
Source: *Table 2B, ISTA rules 2023, p. 2-3*

- Note: Up to including 15 containers, regardless of their size, the same number of primary samples is taken from each container

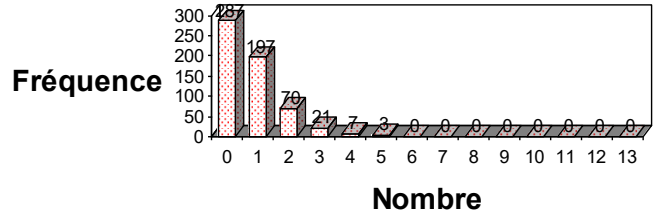
Preparation techniques for the submitted sample - a comparison

- Example: 400 g maize seed received as submitted sample; containing 20 seeds of Sorghum, working sample of 100 g
- Different preparation => differences in frequency of Sorghum seeds
- Results based on 596 analyses *(ISTA, bulking committee)*

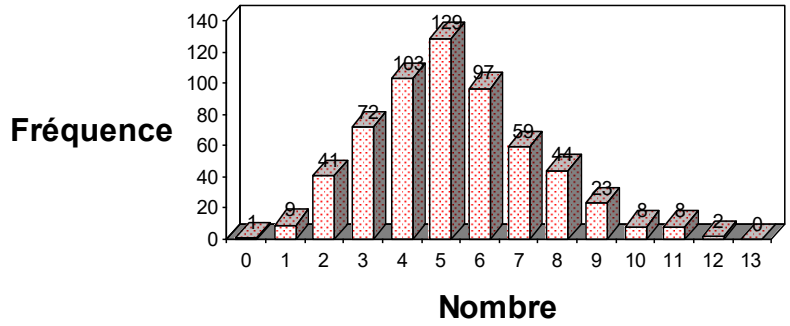
Only weighing



Homogenisations (2x) and weighing



Homogenisations (2x) and divisions with a riffle divider (2x)



✓ Ø = 4,95, counts very close to theoretical distribution laws

Formula to calculate the number of primary samples (1)

- $$\frac{number\ of\ containers\ x\ size\ of\ containers\ (kg)}{100\ kg} = number\ of\ sampling\ units$$
- Example: 170 cartons each containing 24 x 50 g tins
  - (24 tins x 170 cartons) x 50 g / 100 g = 2,04 sampling units
  - Rounding on 3 sample units, then category (1 to 4) with 3 primary samples from each container is appropriate; 3 x 3 = 9
  - Randomly select 9 cartons and take one tin form each carton
  - Divide the seed of the 9 tins with the riffle divider to achieve the submitted sample
  - Replace the 9 tins by additional tins from the same lot or pack the remainder of the composite sample again in tins

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Formula to calculate the number of primary samples (2)

- $$\frac{number\ of\ containers\ x\ size\ of\ containers\ (kg)}{100\ kg} = number\ of\ sampling\ units$$
- Example: 7 bags with 600 kg each
  - 7 x 600 kg = 4200 kg, then category (3’001 to 20’000 kg) with one primary sample from each 500 kg, but not less than 10
  - Divide 4200 kg / 500 kg = 8,4 sampling units, thus less than 10
  - Thus take 2 primary samples from each bag = 14 primary samples
  - In the case that the composite samples weighs less than the minimum required weight for the submitted sample, you have to take 3 primary samples of each bag = 21 primary samples

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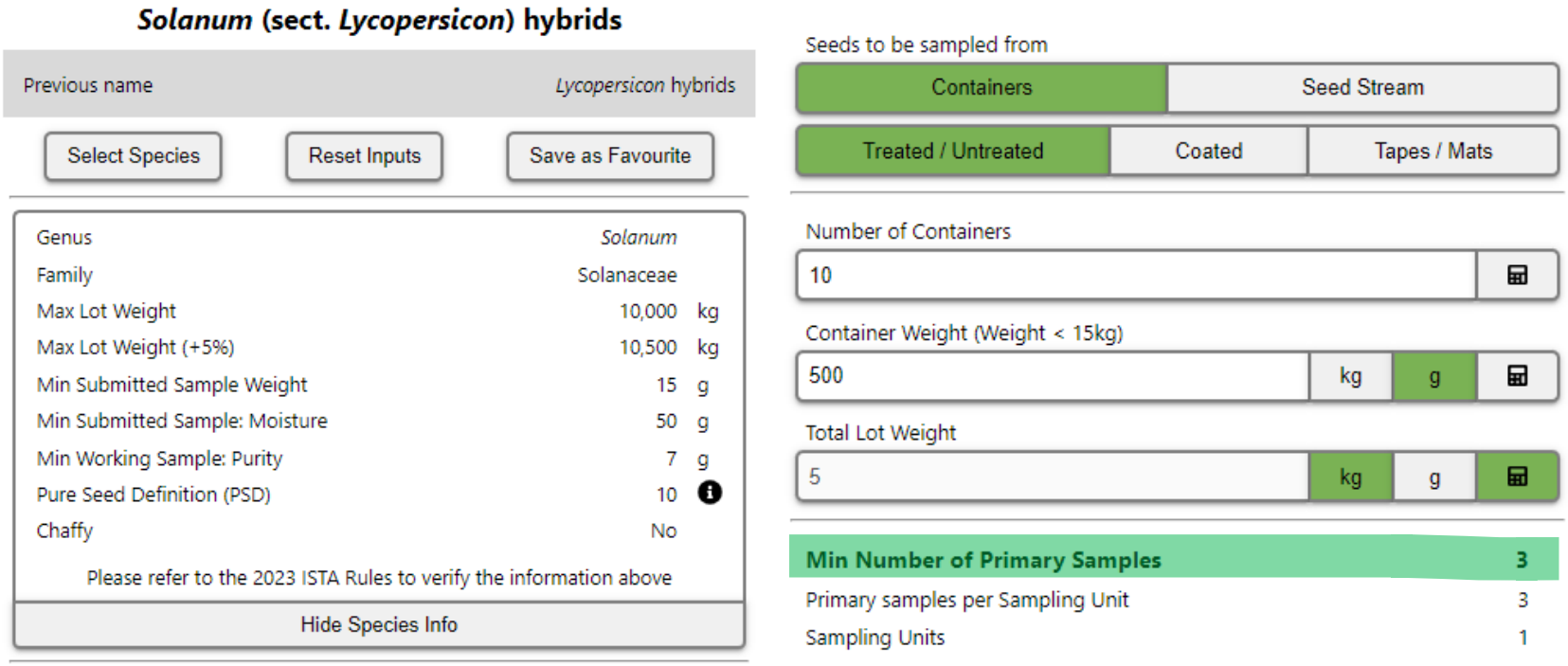
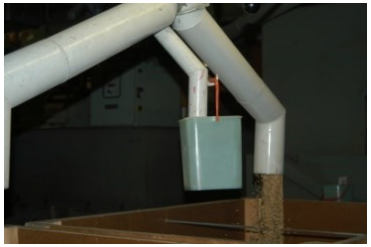
Number of seed sampling units - use of the «seed sampling calculator» tool



Source: [ISTA Sampling Calculator (seedtest.org)]

Sampling methods - by hand, manual or automatic sampling



e.g. Nobbe trier, different stick triers with partition walls for horizontal and vertical sampling

- ✓ Sampling by hand is always allowed
- ✓ must appropriate for chaffy seed (like

Use of the hand halving method


Mixing


Halving (2 port.)


Halve again (4 port.)


Halve again (8 port.)


Separate inner and outer portions


Combine inner and outer portions together

Practical sampling (1)

- Lot of *Phazelia tanacetifola* with 12 bags each with 10 kg
  - Lot size: 120 kg
  - Number of sampling units: 120 / 100 = 1,2
  - Rounding on 2 sampling units

  - Equal distribution of the 12 bags on the two sampling units
  - At least 3 primary samples from each sampling unit must be taken
  - Check with the ISTA-sampling unit calculator number of sampling units

Practical sampling and preparing the submitted sample (2)

- Lot of hybrid Raygrass, variety Pereneia, 2 bags with 12 kg each
- Correct use of the stick trier
- check for heterogeneity
- Correct use of the riffle divider
- Preparing the submitted sample

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Practical sampling - be aware on seed lot heterogeneity (3)

- Sampling of the bag with the stick trier
- Visual check by inspection of the seed distributed in the gutter

Preparing the submitted sample (4)

- Use of the spoon method
- Use the hand halving method
- Use the riffle divider (with or without the attached tipping pan)

- Which method fits well with tomato seeds?

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Thank you for your attention

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