Seed testing: main issues encountered when preparing diagnostic protocols

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Testing for regulated pests

- NPPOs are supporting their inspection procedures for regulated pests with laboratory analysis (both at import export and surveillance of the territory).
- **Reliability of diagnostics is key for NPPOs**
  - Need for validated tests (for 'NPPO labs' in EPPO region guidance on validation is given in PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity*, used for validation in the framework of TESTA).
  - Laboratories increasingly working under quality assurance systems (including validation).
- Currently for NPPOs the result of a test for a quarantine pest is presence/absence.
- Need for a quick laboratory result.
- Seed is a direct pathway.

Key difficulties in testing:
Use of indirect tests: is the pest alive or dead?
Is it pathogenic?

Ideally **viability and pathogenicity should be taken into account when determining the status of a sample** (i.e. infected/non-infected, pathogenic/non-pathogenic).

Main problems:

- Some pests cannot be cultured
- Some are very difficult to culture and culturing will take too much time

Then

- Viability and pathogenicity tests cannot always be recommended. Other tests shown to be reliable are used to determine absence/presence in the sample.
- Whenever possible, p**erforming two tests based on different biological principles** (or for PCR tests targeting different parts of the genome) **is recommended**.
- RNA tests are being developed and when available and practical for routine diagnostics are included.
- PCR findings are interesting information but should be interpreted with care

Key difficulties in testing: Validation with naturally infected seeds

- Not always possible to obtain naturally infected material, spiked samples are used in validation studies (stability of spiked samples is often less good than naturally infected seeds).

Key difficulties in testing: Representative sample?

- Need for more work on sampling both on statistical and epidemiology (e.g. distribution in seed lots)

(cf. presentation on sampling)

Key difficulties in testing: Level of detection and biological relevance

Discussion with seed company experts:

Lots testing positive do not always result in an outbreak/damage in the field. New tests (e.g. real-time PCR) are more and more sensitive and the propagule level detected may not be enough to start a population.

This is a very difficult question and is pest/pathogen dependent and region dependent (level of minimum propagule level may differ). Specific epidemiological studies are needed.
In the absence of such studies it is difficult for plant health regulators and seed companies to agree on a *'safe propagule level'*.

Is there a way out?

- More sharing of knowledge between NPPOs and the seed industry.
- More research on testing and epidemiology possibly joint with NPPOs and the seed industry.
- More international harmonization of seed testing (IPPC Protocols).