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Research for reference

TESTA (Treatment methods, Evidence for Seed Transmission and Assessment of seed health): a European project to study the mode of seed transmission of pathogens and to develop pathogen-detection methods and alternative seed treatments

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Since 2012, GEVES (French Public Interest Group for the Study and Inspection of Varieties and Seeds) and INRA (French National Institute for Agricultural Research)-Angers have been participating in the European project TESTA. This project aims to develop and validate faster, more generic and more accurate methods for assessing seed health. This 40-month project, funded by a €3m grant from the European Union, federates 13 partners (see Box) and seeks to better understand the mechanisms of seed transmission of pathogens, improve sampling and detection methods and assess the efficacy of alternative seed treatments.

Since the 1970s, the commercial seed trade has increased momentously in both volume and frequency and is now global (Figure 1). This expansion in seed trade increases the risk of spread of seed-borne pathogens. Under European regulations, the number of plant protection products available for treating seed is shrinking, with the result that seed health is now critical for limiting the spread of plant diseases.
Many pathogens and pests can be transmitted via seed and methods for assessing seed health must be as generic and as economical as possible. More knowledge on the basic biology of seed transmission of pathogens is needed to continue to protect seed health. Issues regarding seed health must be discussed at the European level as well as internationally and work must be conducted jointly in Europe to guarantee that imported and sown seed is of high quality.
The TESTA project will develop a panel of new methods for assessing seed health and to contribute to the study of transmission of pathogens to seed and from seed to seedlings. The TESTA project will foster the development of appropriate sampling protocols for detecting low levels of pathogens in seed lots, innovative and generic pathogen detection methods, non-destructive and efficient seed disinfection treatments to provide alternatives to the current techniques that use plant protection products that are likely to be prohibited or discontinued in the near future.

Partners in the TESTA project

- The Food and Environment Research Agency (FERA), United Kingdom
- Stichting Dienst Landbouwkundig Onderzoek (DLO), Netherlands
- Institut national de la recherche agronomique (INRA), France
- Universita degli studi di Torino, Italy
- University of Pretoria, South Africa
- Science and Advice for Scottish Agriculture (SASA), United Kingdom
- Aarhus Universitet, Denmark
- National Institute of Agricultural Botany (NIAB), United Kingdom
- Stichting Nederlandse Algemene Kwaliteitsdienst Tuinbouw (NAKT), Netherlands
- Universita degli studi di Modena e Reggio Emilia, Italy
- Groupe d'étude et de contrôle des variétés et des semences (GEVES), France
- Organisation européenne et méditerranéenne de protection des plantes (OEPP), France
- Videometer A/S, Denmark

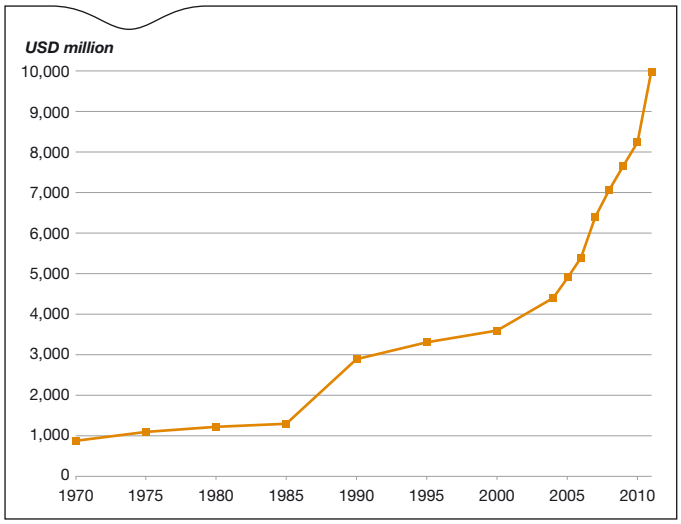

Figure 1. Trends in worldwide commercial seed trade (source: International Seed Federation)

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The project is structured around seven work packages (WPs) (Figure 2):
- seed transmission of plant pathogens
- sampling;
- pathogen detection;
- seed disinfection;
- method validation;
- dissemination of results;
- project management.

TESTA will produce the following output:
- better understanding of transmission of pathogens from seed-bearing plant to seed and from seed to seedling;
- construction of an online database compiling all known seed-transmitted diseases and pests;
- new methods for assessing transmission rates in seed and in crops and associated risk assessments;
- improved sampling protocols;
- new, efficient, generic detection methods;
- non-destructive methods for assessing seed health;
- operational protocols for official testing laboratories;
- seed disinfection methods;
- protocols to assess the efficacy of the disinfection methods.

The Emersys research team at the Horticulture and Seed Research Institute (Institut de recherche en horticulture et semences, INRA-Angers, France) is coordinating WP 1: "Seed transmission of plant pathogens". For this work package, the group will study a panel of phytopathogenic bacteria and their routes and modes of transmission to seed and from seed to seedling.

The pathology laboratory at the French National Seed Testing Station (Station nationale d'essai de semences, GEVES) will carry out the following studies:
- study of the transmission of *Tilletia caries* from seed to seedling and to the soil;
- assessment of the efficacy of hot water treatments for disinfecting alfalfa seed with regard to the nematode *Ditylenchus dipsaci*;
- validation of methods to detect *D. dipsaci*, *Clavibacter michiganensis* subsp. *michiganensis* and *Phoma lingam* in seed;
- organisation of a workshop in 2015 to disseminate results.

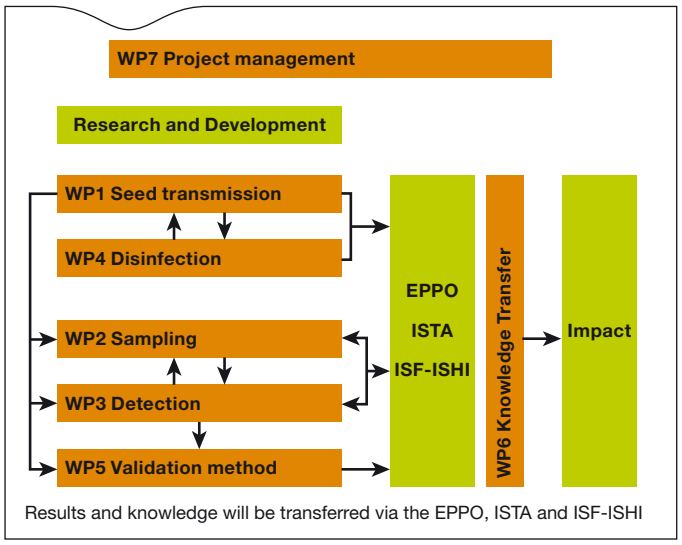

Figure 2. Organisation of the TESTA project.

Figure 3. Extracting the nematode *Ditylenchus dipsaci*

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Transmission of plant pathogens to and via seed

For many phytopathogenic bacteria, seed contamination routes and mechanisms are still unknown. There are three routes through which bacteria can be transmitted to seed: via the vascular system of the seed plant, via floral parts or via contact with contaminated or infected tissues during harvest and threshing operations. Contamination of flowers has been demonstrated to play an important role in the contamination of seed by various phytopathogenic bacteria, including *Xanthomonas* and *Acidovorax* (Darsonval *et al.,* 2008; Lessl *et al.,* 2007). Flower contamination is sometimes also accompanied by internal contamination via the vascular route (Darsonval *et al.,* 2008, 2009). Little information is available on the localisation (internal or external) of bacteria in seed or on how bacteria are transmitted from seed to seedling. Understanding the contamination routes, which determine the future localisation of bacteria in reproductive organs, is essential for selecting new varieties and for screening seeds to avoid or reduce contaminated seed.

Due to the decrease in seed treatments and following the advent of alternative treatment methods, it is not known what levels of seed contamination by *Tilletia* spp. can lead to plant or soil contamination. Although methods for detecting *Tilletia* spp. in seeds have been described (ANSES, 2012), none can rapidly assess the transmission to seedlings or spore viability. The transmission of pathogens to and via seeds will be studied on host-pathogen pairs that have different transmission routes (bacteria in tomatoes, crucifers and cucurbits, fungus and viroids in tomatoes, *Tilletia* spp. in wheat). The following questions will be addressed in this project:

- What is the relationship between the seed contamination rate and disease incidence in the field?
- How does the pathogen enter seed?
- How is the pathogen transmitted from seed to seedling?

The relationship between the localisation of the pathogen in the seed and its transmission from seed plant to seed and from seed to seedling will be studied. This knowledge will help seed companies and public authorities use appropriate methods for disinfecting seed and for detecting targeted pathogens.

Sampling methods

Appropriate seed sampling methods have been developed by international organisations, in particular the ISTA (International Seed Testing Association) and the AOSA (Association of Official Seed Analysts). However, some pathogens are present in seed lots only at very low levels, and their presence – even at such low levels – in a seed lot can lead to high economic losses, or render the seed lot unfit for sale in the case of quarantine pathogens and non-regulated pests that have a severe impact on crops. The best sampling protocol and sample size necessary for detecting the presence of these pathogens have not been extensively researched. The few studies that have been conducted on the distribution of soil pathogens in seed lots (Whitaker *et al.,* 2001) only investigate wheat pathogens.

The TESTA project will use statistical approaches to improve the suitability of the sampling protocol, particularly for large seed lots in which the inoculum level is likely to be low. The sampling protocol developed in the project will be adopted by the ISTA and used by the official testing laboratories and seed companies.

Multi-target detection of pathogens

Detecting pathogens in seed is an important step in assessing seed health to curb the introduction and spread of pathogens in plant crops. The «Seed Health» committees of the International Seed Health Initiative (ISHI), ISTA and the European and Mediterranean Plant Protection Organization (EPPO) have developed, validated and published methods, but each of these methods is for detecting only a single pathogen species.

The purpose of the TESTA project is to improve pathogen detection in tomato, cereal, crucifer and cucurbit seeds by developing generic methods based on multiplex assays and DNA sequencing techniques. DNA/RNA extraction methods and real-time PCR methods will also be improved. In addition, the project will assess new non-destructive methods based on multispectral imaging that can be used on small or high added-value seed lots for which molecular methods are not appropriate.

Disinfecting seed

Commercial seed lots have been treated chemically for decades. However, the number of chemical treatments available has diminished over the past few years. Physical and biological disinfection methods will be developed in the TESTA project. These methods will be based on hot water treatments and microorganisms or natural plant extracts. The ability of microorganisms and plant extracts to control diseases and pests and improve seed germination will be tested. Procedures for assessing the viability of targeted pathogen species will be developed. A guide to choosing the most appropriate disinfection method according to the targeted pathogen will be produced at the end of the project.

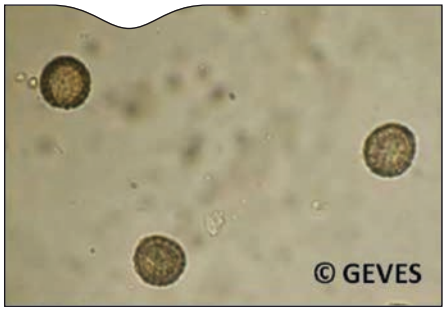

Figure 4. Teliospores of *Tilletia caries*

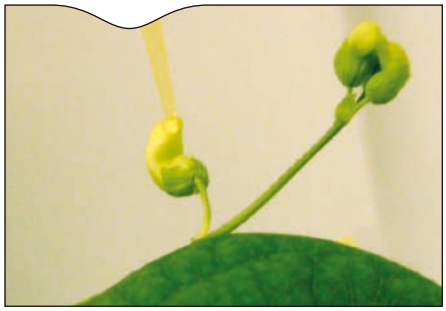

Figure 5. Artificial inoculation of flower buds

Figure 6. Healthy and contaminated lots of bean seed

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Knowledge transfer

The results of the project will be disseminated widely to scientists, public authorities, seed companies and other stakeholders in seed production. The participation of ISHI in the project will ensure that the research undertaken corresponds to the needs of the seed industry and that they are shared with seed testing laboratories. The involvement of ISHI will be instrumental in transferring the developed methods to the seed industry. The information on quarantine pathogens will be disseminated via the EPPO. Training sessions will be provided for seed pathologists and seed quality technicians.

The project deliverables include a database of seed-transmitted diseases and pests, pathogen detection methods for use on seed, an assessment protocol for gauging the efficacy of seed treatments and numerous scientific publications.

Via the TESTA project, new methods and knowledge on seed health will be provided for plant protection services and seed testing laboratories across Europe.

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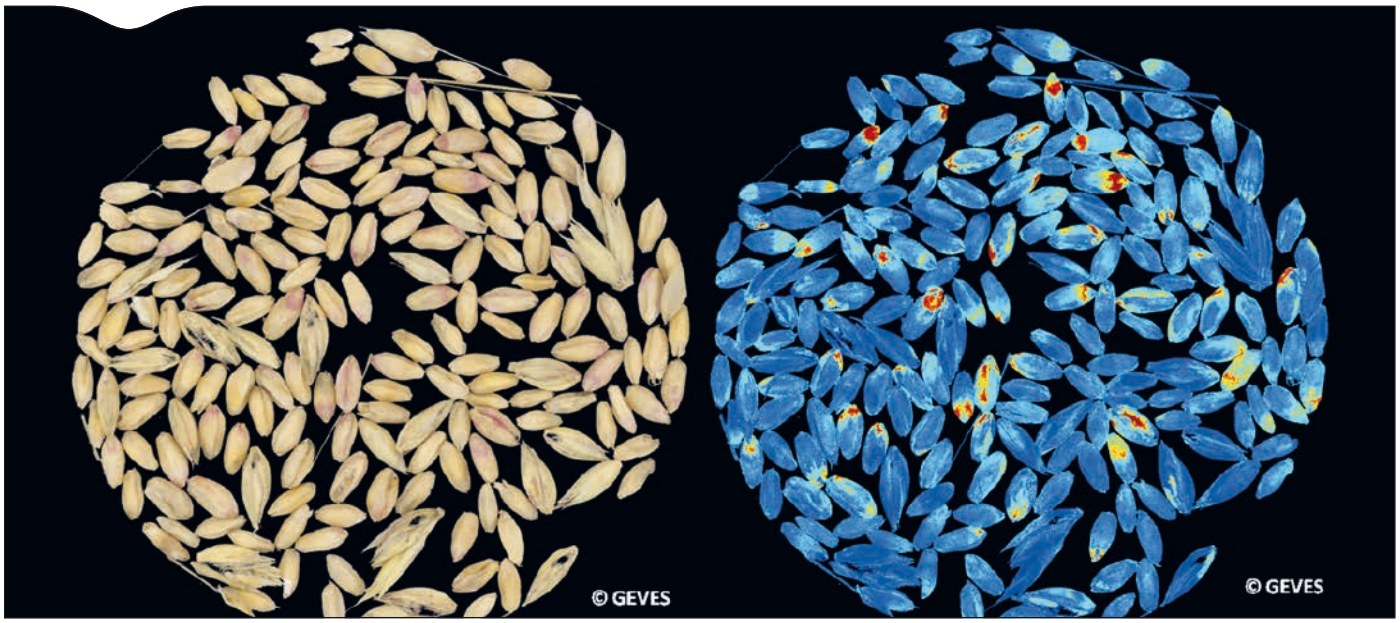

Figure 7. Detection of *Fusarium* sp. in wheat using a VideometerLab instrument.