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Editorial

This first issue of *EuroReference* for 2013 is devoted entirely to the area of plants. As we mentioned before, three issues of *EuroReference* will now be published each year: two biannual issues, plus one special edition focusing on a specific area, like Issue No. 7 in 2012 which discussed the area of safety and security. A wide range of subjects are addressed in this new issue, including regulatory considerations and presentations of networks, joint initiatives and European research projects.

Changes to plant health regulations are driven by National Plant Protection Organisations (NPPOs) to better take into account the European plant health context. Several hundred pests affecting a large number of plant species are regulated in Europe, and given the increasing number of harmful organisms introduced in recent years, classifying pests that are harmful to plant health by priority, using a multi-criteria analysis tool, should enable identification of those that require priority research efforts and development of specific methods, as well as priority management measures in order to optimize plant health. In this issue, you will find an overview of the changes that should be made to the European Union plant health regime, from the point of view of the French Directorate General for Food, and a discussion of the proposed prioritisation methodology for pests in the area of plant health.

Communication between public and private stakeholders is essential to ensure a rapid response in the event of an emerging threat. This is also a good way of optimising resources in view of the challenges facing plant protection. The newly-created French network for plant health (RFSV) and the existing networks (e.g. the European Network of GMO Laboratories - ENGL) should together fulfil this need for communication. This issue of *EuroReference* puts the spotlight on the Q-bacco-net initiative undertaken by the main holders of microorganism collections in Europe, with the aim of facilitating access to biological resources. This is because biological resources underpin all health control processes.

A number of aspects depend on reliability of analytical methods: the quality of health controls, the quality of pest surveillance, monitoring of changes in resistance to plant protection products, and the verification of compliance with regulations that govern import and cultivation of plants, including genetically modified plants. The French procedure for the formalisation of analytical methods in the area of plant health is presented in this issue, in the Methods section.

Reliability is controlled collectively via inter-laboratory proficiency testing. A summary of the tests organised by the laboratories in the region managed by the European and Mediterranean Plant Protection Organization (EPPO) is given in the Agenda section. The lessons learned from nearly 10 years of work in the area are discussed in the Networks section.

Plant protection is an issue that is addressed at the European level. The EPPO (see Focus) is a driving force in this area, and its scope extends beyond the European Union, which remains nonetheless its core region.

Finally, improving plant health management requires enhanced knowledge of the biology of harmful organisms and techniques to identify and detect them. Research is coordinated at the EU level. The EUPHRESCO project is one of the actions intended to support the research efforts in the Member States. The project is presented in this issue. The Knowledge-Based Bio-Economy (KBBE) programme is one of the European Commission's support schemes to reinforce research. The programme's TESTA project, discussed under Research, is an example of an initiative that aims to provide reference laboratories in the area of seed health with new methods and knowledge.

We trust that this issue will be useful to you in your reference activities. We hope you enjoy reading further.

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EUPHRESCO Project – Safeguarding Europe's plant health through research coordination

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EUPHRESCO is a network of European plant health research funders which aims to coordinate national, transnational and EU-funded research in direct support of the Community Plant Health Regime (CPHR). The main achievements and future challenges of the network are presented here.

Introduction

Europe's agriculture, horticulture, forestry and environment are under constant and ever increasing threats from new and exotic plant pests and diseases. Increased globalisation of trade both in terms of volume and diversity, climate change and EU expansion all exacerbate the risks. While these risks grow, our capacity and capabilities to deal with them shrink. Resources for national plant health inspection services, science programmes and research are decreasing. EUPHRESCO (European Phytosanitary Research Coordination) was established in 2006 to help combat these challenges and mitigate the risks posed by pests and diseases through the coordination of plant health research.

History of EUPHRESCO

EUPHRESCO is a network of European plant health research funders which aims to coordinate national, transnational and EU-funded research in direct support of the Community Plant Health Regime (CPHR). The CPHR's primary goals are to prevent the introduction, establishment and spread of regulated and quarantine plant pests through the provision of EU-wide policy, inspection services and science capability. EUPHRESCO aims to better coordinate the European research that underpins plant health policy and its implementation. It will coordinate the research of national plant health programmes and has advised on plant health priorities for EU-funded work under the 7th Framework Programme. By doing so, EUPHRESCO will optimise research funding, promote cooperation, develop common research agendas and foster scientific expertise to improve Europe's phytosanitary capability. The resulting research will underpin plant health policy and regulation to prevent or minimise the risks of quarantine plant pests entry and provide the tools needed for surveillance and for the management of these pests if introduced.

EUPHRESCO began as a network of 23 partners in 17 countries, funded by the EU 6th Framework Programme (FP6) in 2006 (EUPHRESCO-I). Its partners were leading organisations involved with funding phytosanitary (statutory plant health) research in Europe. Expert advice was provided through formal links to European bodies, the European and Mediterranean Plant Protection Organisation (EPPO), the European Food Safety Authority – Plant Health Panel (EFSA-PHP) and the European Commission's DG SANCO.

5 projects were commissioned from the virtual common pot call
- DEP – Detection and epidemiology of pospiviroids
- AMBROSIA – Strategies for *Ambrosia* management
- ERWINDECT – Diagnostic tools for the detection of fire blight
- PROPSCAPH – Risk of spread of *Scaphoideus titanus*, vector of GFDP
- PEKID – Phytosanitary efficacy of kiln drying

2 projects were commissioned from the real common pot call
- QAMP – Whole genomic DNA amplification for quarantine pests
- DECLAIM – Management for invasive aquatic macrophytic weeds

4 non-competitive projects were initiated; these involved the validation of diagnostic methods for regulated plant pests or pathogens
- Potato ring rot and brown rot
- Whitefly-transmitted viruses
- Potato cyst nematodes
- Maize bacterial blight

A second round of non-competitive projects were initiated in 2009/10
- Dickeya ecology and diagnostics
- *Gibberella circinata* diagnostic seed methods
- *Anoplophora detection* and risk management
- Meloidogyne detection and risk management
- Phytoplasma diagnostics (link to COST Action)
- Phylogenetic identification of quarantine bacterial pathogens

Figure 1. EUPHRESCO-I projects

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Main achievements of EUPHRESCO-I

The project's main achievements up to 2010, were:

(1) the mapping and analysing of 46 national plant health research programmes (35 from EUPHRESCO countries and 11 from non-partner countries in Europe and the EPPO region) resulting in a report which identifies gaps and opportunities that could be addressed through transnational research coordination and collaboration.

(2) development of tools and processes for three separate funding mechanisms:
- real common pot: countries provide funds in a single bank account, the best projects resulting from an open call are funded regardless of the nationality of the eligible researchers involved; there is therefore a trans-national flow of funds,
- virtual common pot: each country participating in a call pays only for the involvement of its own researchers in projects resulting from an open common call and,
- non-competitive mechanism: a science/research problem or topic area is divided between research groups organised in a consortium, in different countries according to their expertise; each country pays/provides its own researchers to deliver work to the consortium. There is no trans-national flow of funds; there is no competition (for implementing transnational research);

(3) testing these mechanisms through the commissioning of 11 transnational pilot projects in a 'learning-by-doing' exercise. The funding committed across the 7 competitively-let projects was €1.5 million, relating to 8–10% of the national annual budgets. The 4 non-competitive projects provided a significant amount of additional funding. A further 6 projects commissioned via the non-competitive mechanism were initiated in late 2009 and early 2010 (Figure 1);

(4) advising the European Commission on plant health research priorities in its 7th Framework Programme under a mandate from the EU Council Working Party of Chief Officers of Plant Health Services (COPHS). EUPHRESCO's advisory role has contributed to key strategic topics being included in FP7 calls and projects emerging on the science of pest risk analysis (PRATIQUE Project), DNA barcoding methods for quarantine pests (QBOL Project) and developing field-based detection tools for use by plant health inspection services (Q-DETECT Project);

(5) developing a common strategic research agenda.

The final achievement to highlight is

(6) the development a modus operandi for a strong, long-term and self-sustainable EUPHRESCO network.

EUPHRESCO-II

Since the end of EUPHRESCO-I, the network has continued within a second project funded by the EU 7th Framework Programme; it started in January 2011 and will run till March 2014. This aims to continue and enlarge previously successful cooperation and ensure the consortium will continue after 2014 as a self-sustainable long-term network of European phytosanitary research funders. EUPHRESCO-II will 'deepen' cooperation by continued transnational research that optimises limited resources, supports other plant health initiatives and further improvements of processes and tools and reduced barriers to collaboration. The network has enlarged to 31 partners in 22 countries with 14 observers (Figure 2, maps 1 and 2). Further, it has enlarged its sector coverage and now includes forestry health and has increased opportunities for cooperation and collaboration with non-European countries.

Ten projects, all commissioned via the non-competitive route were initiated in late 2011 and early 2012. The funding

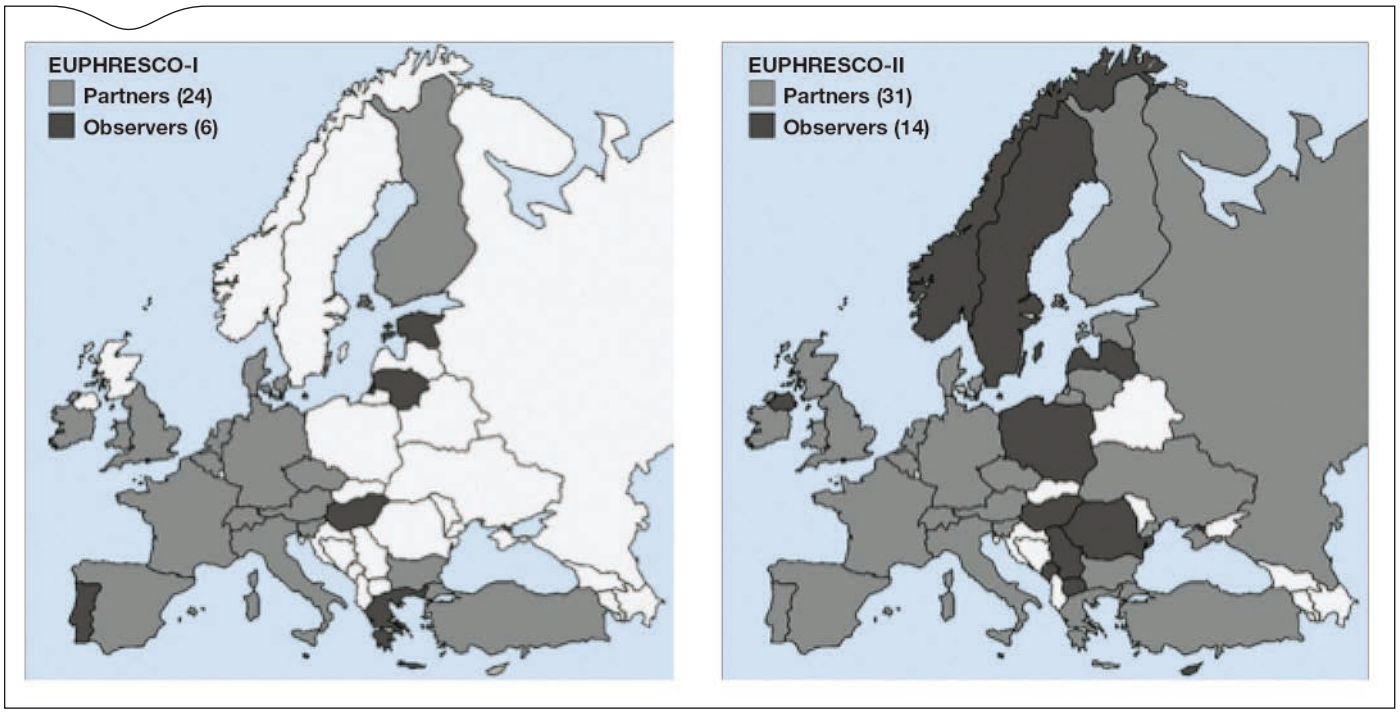

Figure 2. Maps of partners and observers of EUPHRESCO-I (2006-2010) and EUPHRESCO-II (2011-2014).

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committed across these projects was in excess of €2.8 million. The projects covered work on emerging phytophthoras, Potato cyst nematodes, *Meloidogyne enterolobii*, *Synchytrium endobioticum*, pospiviroids, potato phytoplasmas and *Candidatus* Liberibacter *solanacearum*, Grapevine flavescence dorée, fruit tree phytoplasmoses, fire blight, Drosophila suzukii.

Future: the EPPO role in a sustainable EUPHRESCO network

At the EPPO Council Session in 2011 a request was made by some EPPO member countries that the EPPO Secretariat could provide the structures for a long-term sustainable network of EUPHRESCO. There was unanimous support for this suggestion at the last annual EUPHRESCO meeting in 2012. In particular, it was underlined that one of the core functions listed in Article V of the EPPO Convention is to "facilitate cooperation in research on pests and the methods of control and in the exchange of relevant scientific information". EUPHRESCO members also considered that EPPO has the technical capacity to manage EUPHRESCO's research identification and facilitate research coordination, collaboration and cooperation in particular because:

- EPPO has experience in coordination and administration of international groups;
- EPPO has experience in organizing workshops, etc.;
- EPPO's Information Technology expertise and infrastructure;
- EPPO has links to Regional Plant Protection Organizations in other parts of the world, which could be helpful in extending the network;
- EPPO combines many members with a broader scope than the original EUPHRESCO;
- EPPO members are national plant protection organizations with a good background in plant health issues;
- EPPO and its members are important stakeholders of plant health;
- for some EUPHRESCO members it could be easier to give fees (e.g. membership fees for the network) to an international organization such as EPPO rather than to a national organization from another country;
- it could provide the opportunity to include new partners in EUPHRESCO.

Consequently the possibility that the EPPO Secretariat could provide the structures for a long-term sustainable network of EUPHRESCO is currently under evaluation in particular regarding the possible financing mechanisms to fund the co-ordinator position which will be needed in the secretariat. Given the broad support of EPPO members to this request and the interest expressed from the European Commission that this ERA-Net should continue, it is hoped that a positive decision will be made at the next EPPO Council session in September 2013.

It is beyond doubt that the EUPHRESCO network cultivates the optimum environment for concerted research efforts, as it provides a coordinated and cohesive framework within which the science needed by policy makers and inspection services can be developed in this vital field.

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Developing a methodology for the prioritisation of pests in plant health

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With the aim of optimising resource allocation for the prevention, surveillance and control of pests, the authorities in France have chosen to develop a tool to classify pests by priority. This tool has been developed by the ANSES Plant Health Laboratory. The method involves evaluation of the invasiveness of pests that are absent or of limited distribution in mainland France. To this end, multiple criteria are used in an evaluation method based on the principles of pest risk analysis. Using a semi-quantitative model rapidly provides a classification of pests. The tool is available *via* an intuitive IT interface, facilitating use and interpretation of results. For most of the top-ranked pests in the current classification, the Plant Health Laboratory has suitable analytical methods for their detection. However, the classification also singles out pests that deserve specific attention. The aim of this article is to present the regulatory framework of the study in more detail, and to describe the principles underlying the prioritisation methodology.

Purpose of classifying pests by priority in the area of plant health

To promote the reactivity and competitiveness of the agricultural sector in France in a context of globalisation of trade, climate change and evolution of agricultural practices, France modernised its health policy strategy following the National consultation on the health sector of 2010. Priority classification of pests in plant health is part of these efforts. One of the main objectives of the new scheme is to optimise management and funding of health policy. With this in mind, risk managers aim to set up priorities for allocation of the available resources for prevention, surveillance and control activities based on the seriousness of the health risk. This is precisely the purpose of the French Ordinance of 23 July 2011, which provides for classification of health hazards. This requires that pests that threaten plant health be divided into three categories with decreasing degrees of danger, 1, 2 and 3, with the associated funding being the responsibility of either administrative authorities and/or private organisations. In order to establish the necessary categorisation of health risks, the Ministry of Agriculture addressed a formal request to the Agency for Food, Environmental and Occupational Health and Safety (ANSES) concerning development of an objective and transparent prioritisation methodology adapted to the specific biological risks that threaten plant health.

Specific characteristics of biological risks in plant health

The wide taxonomic range of pest organisms, i.e. viroids, viruses, phytoplasmas, bacteria, fungi, nematodes, arthropods and plants, along with the extremely high number of plant hosts, poses a real challenge to the development of a general prioritisation model. Fortunately, an invariable biological principle mitigates this apparent complexity: regardless of the host-organism interaction in question, the risk level for plant health is always dependent on certain key factors for the development of pests. As a result, the prioritisation method involved setting up a classification based on evaluation of these factors that are common to all pests, in the context of mainland France.

Pests of interest for the development of a prioritisation method

Pests inherent to international trade

Globalisation of trade is recognised as a major factor contributing to the introduction and spread of species outside their indigenous distribution area [1]. Import of living plants and plant products from other countries is a key entry pathway for exotic pest species. The greater volumes of imported products and their increased frequency, as well as the cryptic life stages of pests, hinder systematic interception by the health control services [2, 3]. Among these accidentally introduced pests, some prove to be invasive with a negative impact on the health of crops and/or wild plants [4]. More specifically, they may result in economic losses, such as reduced agricultural yields and eradication costs, or undermine the natural ecological balance, or even become a concern for public health [5]. The total economic impact of exotic species in Europe is very roughly estimated to be about 10 billion euros annually [6].

Pests subject to regulatory phytosanitary measures

To prevent introduction and spread of alien pests that pose a risk to plant health, the European Union has implemented specific regulatory provisions. As part of this framework, Directive 2000/29/EC lists several hundred regulated organisms and potential host plants and plant products for which introduction and spread are strictly prohibited. This European directive was transposed into French law by the Ministerial Order of 24 May 2006 concerning health requirements for plants, plant products and other items. Its application involves implementation of mandatory prevention, surveillance and control measures, regardless of the level of phytosanitary risk.

In parallel to the European regulatory context, the European and Mediterranean Plant Protection Organization (EPPO) under the authority of the International Plant Protection Convention (IPPC), recommends that pests be considered regulated

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organisms in national regulations, where member countries consider this appropriate. These organisms are included in two distinct lists designated as List A1 and List A2. List A1 organisms are entirely absent from the EPPO region, while those in List A2 are found locally. The EPPO has also set up an Alert List that contains pests with invasive properties and for which surveillance is strongly recommended. In France, organisms on the EPPO Alert List are mandatory control organisms under certain conditions since their inclusion further to revision of the Ministerial Order of 31 July 2000.
Currently, operational implementation of these regulatory texts is hindered by inadequate resources available in view of the large number of regulated pests. Certain publications propose a list of the top 10 bacterial, fungal and viral agents in terms of risk for plant health based on their scientific and economic importance worldwide [7-9]. These approaches are however not sufficient to prioritise management actions for regulated organisms nationally. As a result, the prioritisation methodology developed here involves pest that are alien, absent or of limited distribution in mainland France which are currently managed on a regulatory basis, and for which it is necessary to evaluate and compare their invasive potential and the impact they represent to wild plants and crops.

Basic principles underlying the development of a prioritisation method for pests in plant health

A method based on procedures for pest risk analysis

To assess the risk related to organisms inherent to the trade in plants and plant products, the reference text is the FAO's standard on Pest Risk Analysis (PRA) [10]. This text harmonises assessment of phytosanitary risk related to organisms that are absent or of limited distribution in a given region to provide all the justifications required for the implementation of regulatory measures that may restrict international trade. Once the geographic area of study has been determined, the PRA uses a questionnaire to determine both the probability of its exposure to an alien organism and the extent of potential negative impacts. The probability of exposure of a given region to an alien organism takes into account the probability of entry, establishment and spread. At the same time, the assessor specifies the degree of uncertainty concerning the risk assessment in view of the available data. When the phytosanitary risk is considered unacceptable, management measures are listed and evaluated. The prioritisation method follows the general structure of the PRA to evaluate the phytosanitary risk.

Biological invasion as the common factor

Beyond its regulatory application, PRA is a method recognised for the way it addresses the concept of biological invasion of alien species [11]. Recently, this concept was formalised to attempt to impose a unified framework on the way it has been applied over the past 20 years [12]. The authors identify four successive stages in describing the process of biological invasion (Figure 1). The fulfilment of each of these stages is dependent on the organism successfully counteracting a wide range of biotic and abiotic forces. The first stage is transport of the organism that enables it to cross biogeographic barriers that would naturally be impassable. A second stage can involve the organism being maintained in a controlled environment (captivity or cultivation). However, in plant health, entry of a pest is primarily accidental with direct passage from stage 1 (transport) to stage 3 (establishment). The PRA standard groups together stages 1 (transport) and 2 (maintenance in a controlled environment) into a single stage called "entry" [10] (Figure 1). Once present in the environment, a local viable population can be established with individuals multiplying and adapting to new conditions. The fourth stage is characterised by spread over a wider area after reproduction of the established population. In this model, the authors do not take account of impacts, considering that they do not determine the invasive nature of an organism.

Characteristics of the prioritisation method

A multiple criteria approach

Unlike PRA, the prioritisation method developed here has the added feature that it generates a classification of pests based on the assessment of phytosanitary risks. As a result, the general structure of the method revolves around "criteria" that characterise the phytosanitary risk of pests. These criteria were defined by adapting the questions in the PRA and by consulting experts. Criteria must differentiate the invasiveness capacity and impact of pests effectively from one another. In the end, 24 criteria were selected and organised into five metacriteria, three corresponding to biological invasion stages, and two concerning impacts (Figure 2). The chosen criteria are often found to be indirect indicators for which data are available rather than variables measuring the phytosanitary risk directly. For example, the volume of import of plants and plant products is regularly used as an indirect indicator of the flow of potentially associated organisms [11].

Semi-quantitative evaluation of criteria

In the PRA model, the assessor measures the components of biological invasion based on a qualitative scale with the following terms: "very unlikely", "unlikely", "moderately likely", "likely", "very likely", and related uncertainty with the terms "low", "moderate", and "high". Although this approach is pragmatic, the final result of the phytosanitary risk assessment is expressed in the form of summaries that are sometimes complex. In the framework of the European research project PRATIQUE, the EPPO has developed a tool enabling conversion and aggregation of qualitative measurements into a probability of entry, establishment, spread and impact [13]. For a given PRA, this innovative assessment method facilitates overall understanding of phytosanitary risk. However, this tool does not make it possible to rank several pests for which the risk was evaluated using PRAs carried out independently from one another.
With the aim of developing a simple operational tool that can prioritise the numerous pests based on multiple criteria, the evaluation method retained here is a semi-quantitative model. This approach has been applied in several models of priority classification of invasive species [14]. The principle is to evaluate heterogeneous criteria by attributing numerical scores to quantify the level of risk. In this way, despite the diversity of criteria, they are aggregated using a single mathematical formula. In the method of prioritisation developed, the scores are between zero for the absence of information, and a maximum score for a major risk. Aggregation of criteria for the same metacriteria is cumulative, while aggregation of entry, establishment, spread, and impact metacriteria is multiplicative. The overall phytosanitary risk index calculated in this way is therefore not only consistent with the notion of phytosanitary risk explained above, but also determines the classification rank of the pest.

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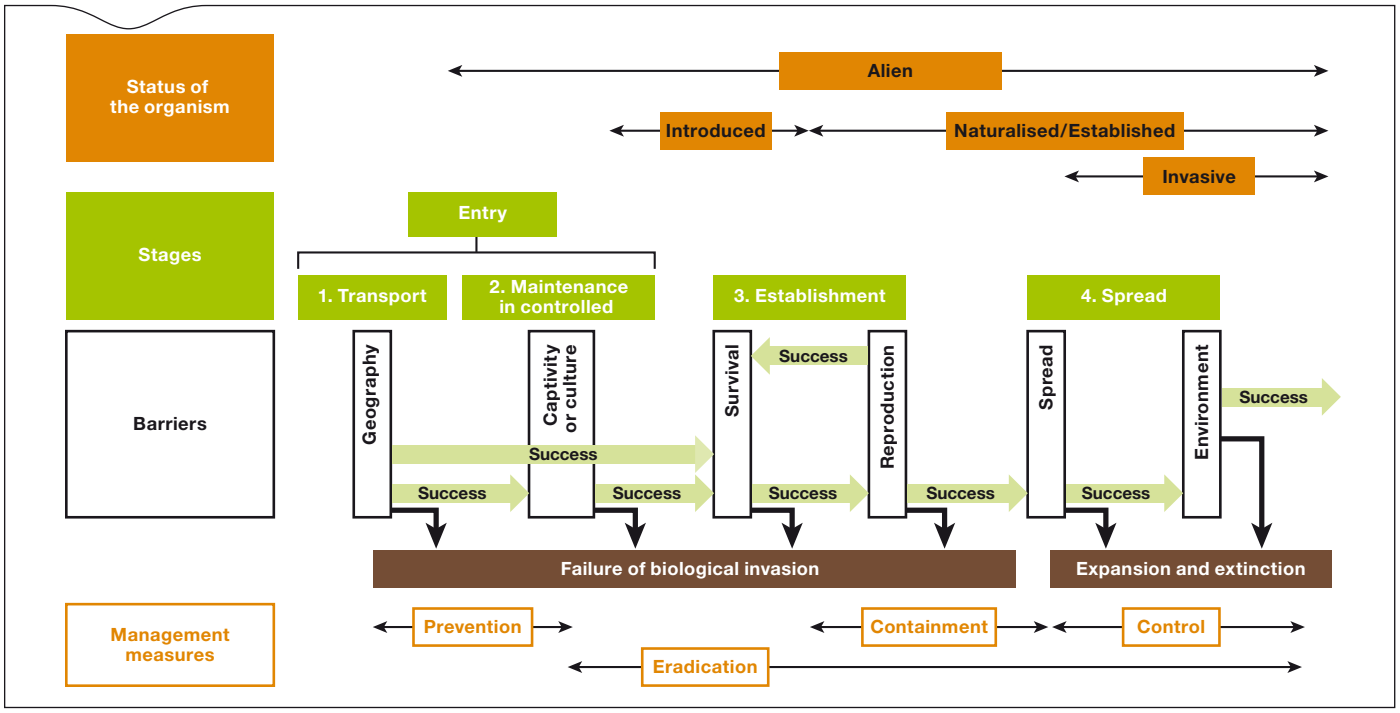

Figure 1. Concept of biological invasion formalised by Blackburn.
An organism is considered invasive in a novel area of introduction once it has overcome several barriers during the four successive stages. In plant health, entry of pests is primarily accidental with direct passage from stage 1 (transport) to stage 3 (establishment). The PRA standard groups together stages 1 (transport) and 2 (maintenance in a controlled environment) into a single stage called "entry" [10] (adapted from Blackburn et al.*, 2011).*

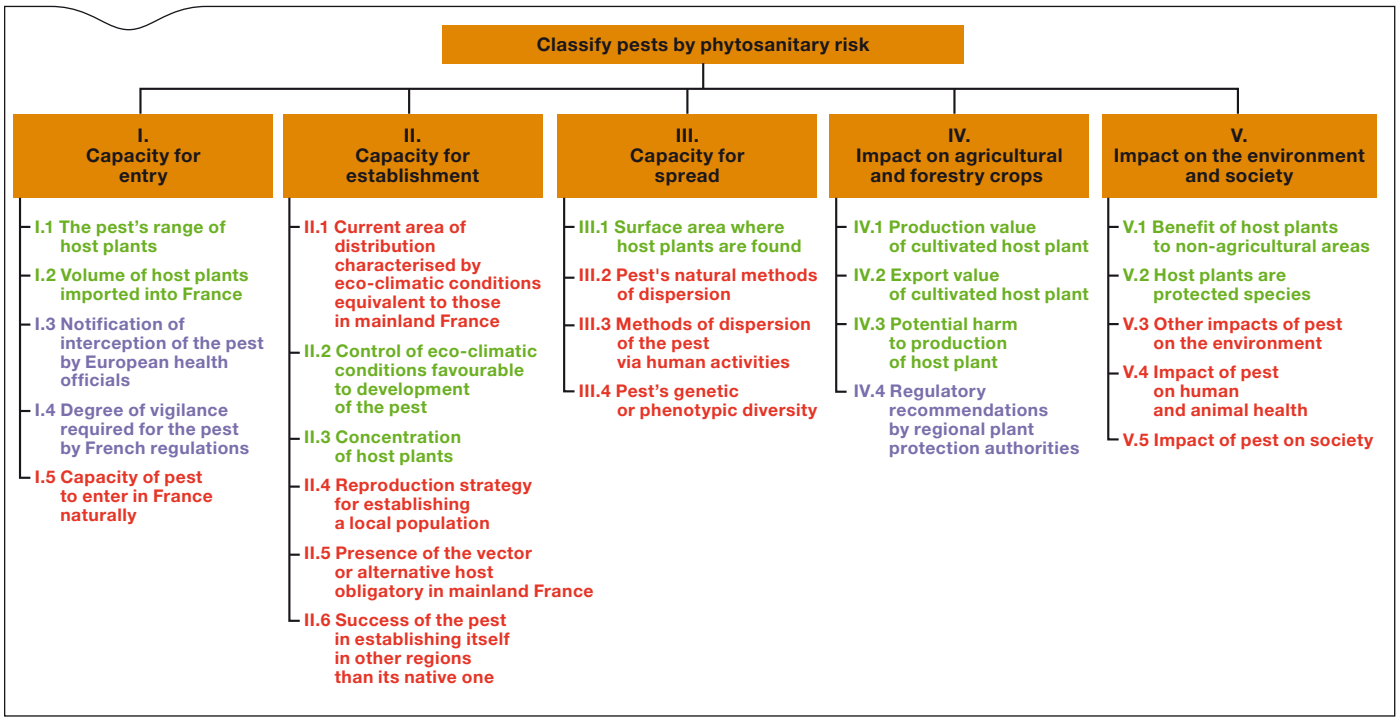

Figure 2. Diagram of metacriteria (orange blocks) and criteria (in colour) selected for the prioritisation method.
The red, green, and purple titles refer to the biology of the organism, host plants, and regulatory measures, respectively.

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A criteria evaluation system based on prior analysis of available data

In the prioritisation model developed, attribution of scores for each criterion is correlated with selection of predefined risk classes. The usefulness of selecting explicit risk classes rather than attributing a score between two values is that it retains consistent grading between the different pests evaluated, but also between different assessors. The clarity of the criteria descriptions and the risk classes was given special attention to limit differences in interpretation of meaning. This is why four to five classes of increasing risk were defined for each criterion. For example, for the criterion "Host plant range", four classes of increasing risk were defined: (1) the host plant for the pest is a single species; (2) the host plants for the pest are species belonging to the same genus; (3) the host plants for the pest belong to several genera within the same family; and (4) the host plants for the pest belong to several families. For quantitative criteria such as volumes imported, production areas, and production and export values, specific statistical data were collected in advance for as many reference host plants as possible. On the basis of this set of data, five statistical classes of equal size were established for each of the criteria. The risk classes then correspond to those statistical classes.. The purpose of this approach is to discriminate between the attributes of the classes in a consistent manner. In addition, the assessor can easily select the class corresponding to the available data.

A deterministic evaluation of the invasive profile of pests that integrates uncertainty

Given that evaluation of the criteria is based on known data, the prioritisation method is deterministic. Its main advantage is that it highlights the relative differences in invasive capacity of regulated pests. The key point is therefore the robustness of the groups of pests in the classification, rather than the rank in the classification, strictly speaking. Furthermore, this approach requires regular data update so that the classification of pests remains relevant in view of new knowledge described by the scientific and technical community. This is because the aim of the prioritisation method is to provide a structured scientific basis supporting decision-makers and other stakeholders in categorising pests in the area of plant health.

Moreover, during criteria evaluation, the available data may sometimes be contradictory or not sufficiently relevant: this is the notion of uncertainty. Uncertainty is taken into account and evaluated in our method by selecting several risk classes for the same criterion. The scores for the minimum and maximum risk classes selected thus define the limits of an interval quantifying the uncertainty of an evaluation. The greater the interval, the higher the uncertainty of criteria evaluation. The rank in the classification determined on the basis of these intervals makes it possible to single out pests with more uncertain invasion profiles.

A method integrated into an operational and instructive IT system

In order to classify a wide range of pests while building an evaluation of their invasion profile, the prioritisation method was implemented using a computer application functioning in Microsoft Excel®. The advantage of this interface is that it enables automatic aggregation of criteria once all the data have been entered by the assessor. In addition, a macro updates the overall classification as and when a new harmful organism is evaluated. The criteria evaluation procedure was developed with fast and intuitive operability in mind. Therefore, an integrated guide provides details on the criteria evaluation procedures. The clarity of the prioritisation method and the user-friendliness of the IT format have been confirmed by several assessors. As a result, the prioritisation method developed not only enables easy interpretation of results, but also transparent consultation of evaluations through an instructive tool.

The main characteristics of the classification obtained with the prioritisation method

Preliminary results validated by experts

The relevance of the pest classification established using the prioritisation method has been evaluated by experts. To begin, 25 alien and indigenous pests covering all taxa and targeting major plant sectors were selected. The phytosanitary profile of these pests was subsequently qualified by experts as high, moderate or low, with no attribution instructions. The 25 pests were then classified using the prioritisation method. The results showed significant correlation between the rank in the classification and the risk profile as determined by experts. More specifically, the prioritisation method made it possible to identify without ambiguity pests with a high risk profile and those with a low risk profile. For example, *Diabrotica virgifera virgifera*, *Tilletia indica* and *Meloidogyne chitwoodi* classified at the highest rank were considered high risk by the experts, while Aculops fuchsiae and *Pseudomonas syringae* pv. *aesculi* qualified as low risk by the experts were ranked at the lowest level. However, pests with moderate risk profiles were positioned more widely in the classification, such as *Phytophthora ramorum* and *Erwinia amylora*. This result is not surprising given how subjective the term "moderate" is in qualifying risk.

Clear correlation between the rank of a pest and the availability of an analytical method

With the aim of prioritising analytical method development within the Laboratory for Plant Health, it was ascertained whether official analytical methods, EPPO diagnostic protocols and validated in-house methods were available for each harmful organism, alongside evaluation of the criteria described above. This enquiry indicated that the pests for which analytical methods are available were ranked at high levels in the classification. As a result, this finding corroborates the relevance of the current working priorities of the Laboratory for Plant Health, and increases confidence in the prioritisation method.

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Limitations of the prioritisation method

The prioritisation method includes five evaluation metacriteria which are relevant only to pests that have not occupied their entire potential ecological niche in France. In other words, evaluation of the entry, establishment and spread metacriteria is not suitable for pests that are indigenous to the country or naturalised over their entire potential establishment area. As a result, defining the status[1] of the harmful organism in mainland France is an essential prerequisite.

The prioritisation method is based on a semi-quantitative model that includes neither temporal dynamics nor spatial heterogeneity of the biological invasion from an overall country perspective. To compensate for this limitation, several studies propose quantitative evaluation of key factors for biological invasion on the basis of equations that model their evolution over time and in space [11]. Nonetheless, as these authors highlight, this type of approach uses specific complex resources which restrict generalised application.

Conclusion

This pest prioritisation method provides an essential scientific basis for progress in French phytosanitary policy. In addition to categorisation of risks to plant health, the prioritisation method opens up other possibilities. With a view to anticipating phytosanitary risks, this approach provides a valuable basis for identifying pests that require closer risk assessment, and in the longer term, that require specific analytical methods. Moreover, the flexibility of this method means that it can be used in other biogeographical contexts. As such, since the second semester of 2012, an adapted prioritisation method is being deployed in the overseas departments and territories of France, to take account of their island context. Finally, the project is clearly an asset supporting the point of view of France during revision of the plant protection scheme at the European level.

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Lab news

Necessary changes to the Community Plant Health Regime according to the French National Plant Protection Organization (NPPO)

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The Community Plant Health Regime (CPHR) aims to prevent the introduction and spread of plant pests in the European Union's territory. This protection applies to crops and wild flora across all environments (cropland, forests, open spaces, the natural environment, etc.). This regime ismainly based on Directive 2000/29/EC, which in particular includes the provisions of a Community Directive dating from 1977. However, recent decades have seen significant developments in this context:

- an escalation in risk factors (significant increase in international trade, expansion of the European Union, climate change),
- increasing limitations on the competent authorities' human and financial resources,
- a rapidly evolving environment (new global and regional standards, new organisations including the European Food Safety Authority (EFSA), new societal expectations, etc.).

Under the leadership of the French Presidency, in November 2008, the Council of the European Union (EU) concluded that there was a need to revise the current regulatory system. An assessment of the CPHR was conducted from June 2009 to May 2010 and led to the formulation of 15 recommendations that specifically demonstrate the need to modernise the plant health regime through:

- a greater focus on prevention;
- prioritising risks for better targeting;
- enhanced solidarity for identifying the best courses of action on issues important to the Community.

This assessment and resulting recommendations formed the basis for discussion and debate in the Member States and the European Commission, especially within the framework of "task forces" set up in 2010 and 2011 that aimed to achieve a consensus about the desired changes among the 27 Member States.

At the same time, the National consultation on the health sector (EGS) was held in France from January to April 2010 at the request of the Ministry of Agriculture, for the purpose of bringing together the different animal and plant health stakeholders for a joint review of the current situation and prospects for the French and Community health systems. This work led the French National Plant Protection Organization (NPPO) to define ten priorities for the new Community strategy on plant health that are largely in line with the avenues for improvement considered by its European partners:

- determining priority pests based on risks to plant health;
- carrying out general surveillance of the status of plant health within the EU's territory;
- reinforcing the requirements and controls on imports from third countries to prevent the entry and establishment of pests in the EU;
- enhancing the control of intra-community trade to prevent the spread of pests within the EU's territory;

making prevention the central focus of the plant health regime by involving and empowering the professionals;
emphasising economic considerations;
continually adapting regulations to developments in the plant health situation and improving their comprehension;
harmonising and improving the efficacy of inspection practices;
supporting and developing research;
integrating plant health strategy with other EU policies.

Within this context, the French NPPO advocates certain specific changes that it considers necessary for a truly effective Community plant health strategy.

Firstly, reversal of the current EU import strategy is deemed essential for more effective protection of Community territory. This would mean transitioning from the current system, in which everything that is not explicitly prohibited is allowed, to a system in which, at least for new trades of plants for planting, a prior pest risk analysis (PRA) should be conducted before imports can be authorised. Imported plant products would thus be subject to more stringent requirements that would be above all more closely adapted to the risks they pose, ensuring their phytosanitary quality.
Furthermore, the French NPPO favours expanding the scope of the CPHR to cover certain invasive alien plants that are harmful to plants and have a significant economic or environmental impact. In effect, the CPHR should come within the scope of the International Plant Protection Convention (IPPC) and its definition of pests in the broader sense ("Any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products").

Finally, the French NPPO considers it necessary to move to a system of prior accreditation of institutions that put into circulation plants subject to an EU plant passport (PP) and that are authorised to self-print PPs. This accreditation would be issued after verifying the institution's competence, especially with regard to its internal risk management system (good practices, traceability, internal controls, etc.). This is because facility inspections within the framework of the PP should include not only an examination of materials present on the day of the inspection (current PP control), but also and more importantly, a second level control that verifies the facility's orderly conduct, i.e., plant production conditions, in order to prevent plant health problems. The frequency of these inspections would be adapted to the type of institution and the risk analysis carried out by the plant protection authorities, i.e. in particular, based on a formal pest management plan established by the business itself. This accreditation system would lead to greater efficiency of official controls and a higher level in the overall phytosanitary quality through greater company accountability.

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Because of the substantial burden anticipated for small businesses (especially the ornamental plant sector), operators would have the option of not joining this accreditation system, and in this case they would be subject to tighter official controls. In all cases, however, the businesses, whether or not they are authorised to issue their own PPs, should comply with general requirements of internal pest risk management to be defined at the Community level, which should go beyond mere traceability requirements for plant products.

These are the avenues for improvement the French NPPO hopes in the new European regulation, whose formal proposal by the European Commission is expected in 2013.

The French Plant Health Network (RFSV): a new tool for protecting plant health

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The national consultation on the health sector (Etats généraux du sanitaire) organised by the French Ministry of Agriculture in 2010 demonstrated the strategic importance of managing crop and forest health in order to reconcile the economic and environmental challenges of our agriculture sector.
With this in mind, the Directorate General of Food (DGAL) asked various bodies (ACTA – the Network of agricultural technical institutes, ANSES – the French Agency for Food, Environmental and Occupational Health & Safety, INRA – the National Institute for Agricultural Research, and UIPP – the French Plant Protection Industry Union, which together make up the secretariat) to set up the new French Plant Health Network (RFSV). This network, which met for the first time in October 2011 at the initiative of ANSES, has grown rapidly since then and now boasts around a hundred members. Its role is to help improve knowledge of plant health.

For this purpose, the RFSV's mission is to foster research partnerships between public and private sector players, throughout the system, from the field to the testing laboratory. It seeks primarily to enhance diagnostic capabilities, while also investigating bioaggressor control methods.

On this basis, the network has identified priority objectives and ten working groups have been set up to address a variety of topics:

- Improving comprehension of the available offer in terms of analysis, skills and research; directories are to be compiled and compared to actual needs. A scheme for skills development is also planned.
- Identifying needs in terms of new laboratory analytical methods and pest control methods; innovative tools and initiatives will be proposed and tested. It will also be necessary to establish channels for transferring methods from public and private research laboratories to routine laboratories.
- Improving knowledge on the evolution of bioaggressors and their characterisation, as well as plant resistance and tolerance to them. Knowledge will also be developed in epidemiology, based on laboratory and field data.

For further information: www.rfsv.fr

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Focus on a laboratory

The European and Mediterranean Plant Protection Organization, one of our objectives: serving the needs of plant pest diagnostic laboratories

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EPPO is an intergovernmental organization responsible for European cooperation in plant health. The objectives of the organization are to protect plant health in agriculture, forestry and the uncultivated environment, to develop international strategies against the introduction and spread of dangerous pests, to encourage harmonization of phytosanitary regulations and all other areas of official plant protection action and to promote safe and effective control methods. The different activities conducted in this framework are presented.

Introduction

EPPO is an intergovernmental organization responsible for European cooperation in plant health. Under the International Plant Protection Convention (IPPC) EPPO is the regional plant protection organization (RPPO) for Europe and the Mediterranean region. Founded in 1951 by 15 European countries, EPPO now has 50 members (see Figure 1) covering almost all countries of the European and Mediterranean region. National Plant Protection Organizations are the EPPO contact points. The objectives of EPPO are to protect plant health in agriculture, forestry and the uncultivated environment, to develop international strategies against the introduction and spread of dangerous pests, to encourage harmonization of phytosanitary regulations and all other areas of official plant protection action and to promote safe and effective control methods. As a Regional Plant Protection Organization, EPPO also participates in global discussions on plant health organised by FAO and the IPPC Secretariat. More information on the Organization is presented in Box 1.

One of the main aims of EPPO is to help its members to prevent entry or spread of dangerous pests. The Organization has therefore been given the task of:

- identifying pests which may present a risk for the region (early warning),
- evaluating their risk for the region and making proposals on the phytosanitary measures which can be taken against them (Pest Risk Analysis).

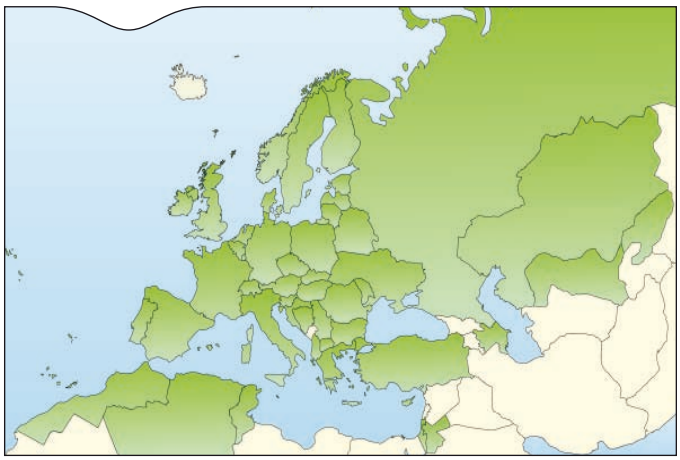

Figure 1. Map of EPPO member countries

Once a pest has been evaluated and countries have agreed that it should be added to the EPPO Lists of pests recommended for regulation, recommendations on how to detect and identify the pest may be developed (diagnostic protocols and phytosanitary procedures for inspection) as well as recommendations on how to eradicate and control this pest. In addition to pest specific activities, EPPO has also developed recommendations for quality assurance in laboratories, in order to promote harmonization of procedures in the EPPO region. To perform these activities, much information on pests presenting a phytosanitary risk to the EPPO region is required and is collected by the Organization and made available to its members. Different databases have been developed including PQR (Plant Quarantine data Retrieval system) and the EPPO database on Diagnostic expertise. the different activities conducted in this framework and that are of interest for plant pest diagnostic laboratories are presented.

EPPO activities serving the needs of plant pest diagnostic laboratories

Early warning

The EPPO Secretariat has established early warning systems to identify emerging risks:

- The Alert List draws the attention of EPPO member countries to certain pests potentially presenting a risk to them. The Alert list is updated regularly: http://www.eppo.int/QUARANTINE/Alert_List/alert_list.htm.
- A free monthly newsletter (EPPO Reporting Service) is published containing information gathered from National plant protection organizations, literature and internet surveys: http://www.eppo.int/PUBLICATIONS/reporting/reporting_service.htm
- The List of invasive alien plants to be managed as a priority in EPPO member countries. This list is established on the basis of a prioritization process: http://www.eppo.int/INVASIVE_PLANTS/ias_lists.htm#IAPList

With such lists, laboratories can be alerted on potential new pest for which they may have to develop and/or validate diagnostic tests.

Evaluation of potential risks: Pest Risk Analysis

Measures adopted by countries to protect their territories from the introduction of new pests should be technically justified.

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The organization of EPPO

EPPO is administered by an Executive Committee (seven countries elected on a rotational basis, meeting twice a year), under the control of its Council (representatives of all member countires, meeting once a year usually the Heads of the NPPOs) headed by a Chairman and a Vice-Chairman, elected as individuals.

The Secretariat (permanent staff of EPPO working at the headquarters in Paris) is composed of 13 persons (including 8 scientific staff members). EPPO is financed directly by annual contributions from its member governments. Its official languages are English, French and for certain purposes Russian.

The technical activities of EPPO in the field of phytosanitary measures (also often called "plant quarantine") are directed by the Working Party on Phytosanitary Regulations. This Working Party meets once a year (in June). Meetings are held in member countries throughout the EPPO region. The Working Party draw up its programmes subject to the approval of the Executive Committee and Council and assigns specific tasks to Panels of experts or Expert Working Groups (for one off activities). Panels are composed of specialists from member countries, nominated as individuals by their respective NPPOs, and they prepare detailed draft standards which will be recommended to all member countries (see details of the procedure below). Every year, 20-25 Panel meetings are held in Paris or in scientific centres throughout the region. Panels generally meet once a year, but this can be adapted according to the priorities and work programme of the Organization. The technical work of the Organization depends on the active and continued participation of experts from member countries in the Working Party and Panel meetings.

EPPO's recommendations to its member countries

As a result of the work undertaken by the different technical bodies of the Organization, EPPO makes recommendations to the NPPOs of its member countries (including the recommendation regarding pests that should be regulated). These recommendations are Regional Standards in the sense of the revised IPPC. In order to ensure international acceptance, draft standards go through a complex approval procedure, during which all member countries have the opportunity to express their views. Final decisions are obtained by consensus and EPPO Standards are officially adopted by the EPPO Council. EPPO Standards have been developed within the two main fields of EPPO activity (plant protection products and phytosanitary measures).

All Standards are published in the EPPO Bulletin and are also available on the EPPO website.
http://archives.eppo.int/index.htm

For more information visit the EPPO Website
http://www.eppo.int/

Encadré 1. The organization of EPPO

A system has been established to perform Pest Risk Analysis (PRA) at the EPPO level and Expert Working Groups are convened to conduct PRAs on specific pests. Five PRAs are conducted every year including the identification of possible measures to prevent the introduction of these pests. Experts from laboratories of the EPPO region often collaborate to these evaluations and EPPO is willing to strengthen this participation.

Recommendations on the pests which should be regulated as quarantine pests

Pests which have been evaluated through the EPPO system and have been recommended for regulation as quarantine pests for the EPPO region are included in the EPPO A1 and A2 Lists. EPPO maintains appropriate documentation on the pests included on these lists. From these lists, priorities for the preparation of diagnostic protocols are made.

Recommendations on how to detect and identify pests: diagnostic protocols

The programme to prepare diagnostic protocols for regulated pests of the EPPO region was initiated in 1998. The work is conducted by the different specialized diagnostic Panels and the work programme is overseen by a horizontal Panel (the EPPO Panel on Diagnostics and Quality Assurance). The list of existing EPPO Panels is available at http://www.eppo.int/ABOUT_EPPO/panels.htm. The diagnostic protocols are written by assigned authors according to a common format and are then reviewed by the relevant diagnostic Panels. They are approved following the regular EPPO Standards approval procedure. The first EPPO Standards for diagnosis were published in 2001. More than 100 pest specific Diagnostic Standards have been approved in all pest groups and more than 10 are currently in preparation. The Diagnostic protocols are freely available online: http://archives.eppo.int/EPPOStandards/diagnostics.htm

In order to ensure the quality of diagnosis performed in the laboratories, standards on quality assurance have also been developed and two Standards on quality assurance for plant pest diagnostic laboratories have been adopted :

- PM 7/84 Basic requirements for quality management in plant pest diagnostic laboratories,
- PM 7/98 Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity.

The Standard PM 7/98 is currently under revision to take into account the recent experience of laboratories with validation of tests.

To perform these activities, much information on pests presenting a risk to the EPPO region is required and is collected by the Organization and made available to its members. Different databases have been developed including PQR (Plant Quarantine data Retrieval system) and the EPPO database on Diagnostic expertise. A system to be used by National Plant Protection Organization to communicate pest reports is also under development.

Plant Quarantine data Retrieval system (PQR)

PQR is the EPPO database on quarantine pests. The development of PQR was initiated by the EPPO Secretariat in 1984. The first database appeared in 1990 but was an internal tool for the EPPO Secretariat. It was suggested that it could also be a useful tool for EPPO member countries, and in 1991 the first version of PQR was released to the NPPOs. From 1991

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to 2007, several PQR versions were distributed on disks or CD-Roms to the NPPOs. In April 2007, the Executive Committee agreed that the database should be made freely available on the EPPO website (as a downloadable computer system). The EPPO new version of PQR (5.0) was launched in 2011 a with a largely modified interface to allow more rapid access to the data and 'real-time' update of the contents.

The database is developed and maintained by the EPPO Secretariat. It gives access to data on:
- all the pests of the EPPO A1 and A2 lists and of EU Directive 2000/29 ;
- pests of the EPPO Alert List ;
- plants of the EPPO List of invasive alien plants ;
- many other quarantine pests and invasive plants of interest to other regions of the world (data obtained from FAO, CABI or from other RPPOs, but with less detailed information than for the EPPO and EU pests).

For each pest, it is possible to lists of host plants, commodities able to act as pathways in international trade, and details on geographical distribution (including maps). Conversely, it is also possible to obtain specific lists of pests, by stipulating the host species, the commodity, and the country of interest. PQR contains general nomenclatural and taxonomic details on pests and hosts.
At present, PQR contains documented information for more than 1,400 pests. However, as already stated, data is more complete for EPPO/EU listed pests than for other types of pests.
PQR can be freely donwloaded at http://www.eppo.int/DATABASES/pqr/pqr.htm
An online database (web-based interface) is currently under development.

EPPO computerized system for pest reports

In September 2011, the EPPO Council adopted a new Standard PM 1/5(1) Format for pest reports. Since the adoption of this format, several EPPO member countries have started to use it to report their pest outbreaks. The EPPO Secretariat is currently developing a computerized form based on this Standard. In 2012/2013, all EPPO member countries will be invited to use it and provide feed-back. It is also envisaged to initiate technical discussions with the International Plant Protection Convention Secretariat to develop a common XML format Such a format will enable countries to send their pest reports to the International Phytosanitary Portal via EPPO, if they wish to use this possibility.

EPPO database on diagnostic expertise

In 2004, the EPPO Council stressed that the implementation of phytosanitary regulations for quarantine pests was being jeopardized by decreasing expertise in plant protection and declared a state of emergency for Plant Health often referred to as the "Madeira declaration" (EPPO, 2004). Following this declaration, several regional initiatives were taken.
At the EU level a proposal was made in 2005 for a Phytosanitary ERA-Net to coordinate national and regional phytosanitary research programmes which resulted in the establishment of the EUPHRESCO project ('European Phytosanitary Research Coordination' http://www.euphresco.org, (Inman, 2006)).
Another regional initiative was suggested by the EPPO Panel on Diagnostics in 2005. This Panel decided to identify practical actions to improve collaboration on diagnostics in Europe and to provide good scientific support for the diagnostic work of NPPOs. It recommended that the EPPO Secretariat should compile an inventory of the available expertise on diagnostics in the region and of training capacities in diagnostics. The EPPO database on diagnostic expertise came into life. This database provides an inventory expertise available in the EPPO region. Its aim is to cover the expertise on regulated pests (i.e. pests of EPPO A1 and A2 Lists, pests mentioned in EPPO Standards PM4: Production of Healthy Plants for Planting), pests possibly presenting a risk to EPPO member countries (EPPO Alert List) and plants of the EPPO List of invasive alien plants. This database does not include common pests which are widely distributed in the EPPO region. The EPPO Secretariat is maintaining the database but note that all information included in the database is based on individual expert's own declarations of their expertise.
In December 2012, a new section "validation data for diagnostic tests" was added at the request of laboratories which are engaging in an accreditation process. As laboratories preparing for accreditation should only use validated tests it was considered that sharing validation data at EPPO level will save resources and promote collaboration. The data included in the database have been generated by various laboratories in EPPO member countries. The validation data are presented according to a common format developed by the EPPO Panel on Diagnostics and Quality Assurance. Validation data can be submitted by any laboratory registered in the EPPO database on diagnostic expertise.
The database can be visited at http://dc.eppo.int/
Finally, since 1985 EPPO has a regular programme of Conferences and Workshops on diagnotics reports of these different events are available at http://archives.eppo.int/MEETINGS/EPPO_workshops.htm. These meetings are unique occasions for experts to meet to exchange information on the diagnostic of regulated pests.

Conclusion

One of the consequences of the increase in international trade in recent years is that European countries have been faced with the introduction of several new pests (e.g. *Bursaphelenchus xylophilus*; *Drosophila suzukii*; *Tuta absoluta*; *Pseudomonas syringae* pv. *Actinitdiae*...). By providing prompt information and alert to National Plant Protection Organizations and by encouraging harmonization of plant pest diagnostics, EPPO hopes to contribute to the prevention of introduction of new pests from other parts of the world which could damage crops or the environment, and to the limitation of their spread within the region should they be introduced.

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Méthods

French procedure for the formalisation of analytical methods in the area of plant health

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The purpose of this article is to present the procedure followed in France to develop and validate official methods in the area of plant health. It was established jointly by the supervisory ministry (Agriculture) and the National Reference Laboratory (ANSES Plant Health Laboratory) in order to take into account each partner's constraints and objectives. While the procedure remains open to change, specifically so that new method characterisation approaches and new techniques can be integrated, it is now organised around several major phases, each of which is presented below. One of the unique features of this procedure, specific to the field of plant health, is its transparency, which is ensured by external consultation.

Introduction

To protect the national territory against quarantine pests and ensure corresponding surveillance activities in accordance with the EU regulatory provisions in force (specifically Directive 2000/29/EC and its implementation texts), the French State implements surveillance and control plans. Surveillance plans apply to plants and plant products upon import, as well as those already present on the national territory (in nurseries, in the field, etc.). To guarantee the quality of exported products, analyses can also be carried out in the framework of EU plant passports, or with the aim of issuing health certificates for non-EU countries.

The analyses carried out on behalf of government bodies, such as the Directorate General for Food (DGAL), Regional Food Authorities (SRAL) and the Border veterinary and plant health inspection service (SIVEP), are termed "official" analyses. Aside from certain specific cases, these analyses can only be performed by accredited laboratories, National Reference Laboratories (NRLs), or so-called "recognised" laboratories (French Rural and Maritime Fishing Code (CRPM), Article R. 202-8). As the advocate, the Directorate General for Food defines the methods that are to be used for these analyses (Article R. 202-17 of the CRPM). Although the use of alternative methods is possible under the provisions of this article, use of the official methods ensures consistency in the surveillance system and reliability of results supplied by the 20 accredited laboratories that are part of this network in France (see list at the following address (in French): http://agriculture.gouv.fr/la-liste-des-laboratoires-agrees).

The purpose of this article is to present the formalisation procedure for analytical methods in the area of plant health, as it is currently implemented by the ANSES Plant Health Laboratory and the DGAL within the Ministry of Agriculture, Food and Fisheries.

Overall presentation

The definitions of the terms used in this article that serve as a framework for the Plant Health Laboratory are presented in Box 1 - General definitions concerning methods, and in Box 2 – Definitions concerning method performance criteria. The full process for formalising a method in the field of plant health is shown in Figure 1. The four main phases can be summarised as follows:

- determination of requirements, method selection and development;
- method characterisation and intra-laboratory validation (sometimes on an inter-laboratory basis, when needed);
- external consultation for the draft method, including public consultation;
- method formalisation by the competent authority.

These phases are presented below with a focus on the particular features or specificities of plant health compared to other fields of activity.

Determination of requirements, method selection and development

Method development and scientific and technical support to the supervisory body are among the specific missions of National Reference Laboratories, as indicated in the CRPM, Article R. 202-5. As such, the methodological needs for the official analytical purposes of the State are conveyed to the NRL.

Given that official methods must be suitable for their intended use in order to be validated (see below), the preliminary discussions between the NRL and the sponsor are a key phase for the success of any project. During this phase, it is essential that the explicit and implicit requirements of the client, for example the DGAL, be clearly determined.

In the area of plant health, the DGAL's general needs are laid down in a document called "specifications for the validation of official analytical methods", signed jointly by the DGAL and the NRL, which acts as a service provider for method selection and development. While the specifications can be adapted to each case, depending on the specific pest involved, the epidemiological background, the degree of urgency, etc., they form a general framework specifying the criteria for selecting a method depending on its intended use. For example:

- an analytical method intended to support management of an outbreak will be more suitable for its purpose if it provides rapid, cost-effective results. The aim in this case is to obtain results for a large number of samples in a short period of time, in order to delineate the area of infection;
- a method intended for the control of imported plant products to detect a quarantine pest that is not present on the national territory will need to be as sensitive as possible to avoid introducing any such quarantine pest, and will need to provide fairly rapid results to enable batch release of the consignment.

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Méthods

Analytical method
Written procedure describing all the means and operating conditions required to detect and/or [identify] [...] the analyte, including: scope, principle and/or reactions, definitions, reagents, equipment, operating procedures, expression of results, precision, and test report.

Alternative analytical method
Analytical method used by a laboratory instead of a reference analytical method.

Reference analytical method
Analytical method recognised by experts or used as a reference by agreement of the parties that yields, or is assumed to yield, the accepted reference value for the physical quantity of the analyte to be measured.

Official method
Analytical method drafted by the NRL and published in the Official Bulletin of the Ministry of Agriculture, to be used when performing official analyses.

Method evaluation (= characterisation of method performance criteria)
Determination of the values of the performance criteria of the method.

Box 1. General definitions concerning methods

These initial discussions between the DGAL and the NRL therefore aim to lay down specific objectives based on the expected target performance criteria, mainly concerning theoretically acceptable levels of false negatives (sensitivity) and false positives (specificity). However, as the examples above demonstrate, criteria other than technical performance parameters (such as rapidity, costs, timelines, and ease of use) must also be taken into account when defining the suitability of a method for an intended use. The purpose may indeed prompt the NRL to opt for one method over another, particularly since choices often need to be made, and a balance struck between sensitivity and specificity criteria.
Once the objectives and expectations have been defined, the reference laboratory carries out a literature study to determine the state of the art, and then, i) develops a method in-house, or ii) performs an initial comparison of existing methods (scientific publications, etc.), or iii) outsources method development. Once these activities have been completed, the laboratory must have a method that can then be characterised in terms of performance criteria. It should be noted that some data collected during the development phase may serve as a basis for the characterisation report.

Method characterisation and (intra-laboratory) validation

A number of standards propose method characterisation methodologies. Some are relatively general (ISO 16140, ISO 5725, etc.), while others are more technical and specific to the area of plant health (EPPO PM7/98). On the basis of these standards and the specifications established with the DGAL,

Sensitivity (of a method)
Probability of detecting a target organism (positive result) in an infected or contaminated test substance. In other words, the ability of a method to detect the analyte when it is present in the sample.
The concept of sensitivity includes inclusivity and detectability (or analytical sensitivity):
- inclusivity: Ability of the alternative method to detect the target analyte among a large range of strains. It can be expressed as a percentage of detected strains or by the known risk (given the state of knowledge at the time of testing) related to evaluation of target intra-taxon variability;
- detectability: Ability of an alternative method to detect the target analyte in a serial dilution.

Specificity
The degree to which an analytical method concerns only the property or analyte of interest, with the certainty that the result is derived only from the analyte.
In other words, specificity is:
the ability of the method not to detect the analyte when it is not present in the sample;
or the ability of the test to provide a negative result for a healthy sample.
Note: specificity is basically the same as exclusivity: Absence of interference by a suitable range of stains, isolates, populations, etc. that are not targets of the method.

Accuracy
Closeness of agreement between a test result and the accepted reference value. In other words, the number of agreements between the results obtained and those expected, relative to the total number of results.
It includes both the sensitivity and specificity of the method.

Detection limit or threshold
"The lowest concentration or amount of analyte that can be detected [...] in the experimental conditions described in the method". It corresponds to analytical sensitivity.

Repeatability
Closeness of agreement between successive and independent results obtained with the same method, for the same test material, in the same conditions, i.e. equipment, operator, and laboratory, within short intervals of time (repeatability conditions).

Reproducibility
Closeness of agreement between individual test results obtained with the same method, for the same test material, by operators in different laboratories, using different equipment (reproducibility conditions).
A reproducibility test involves analysing the same sample in different conditions. In this case, the coefficient of variation is a simplified expression of the reproducibility of the method.

Box 2. Definitions concerning method performance criteria

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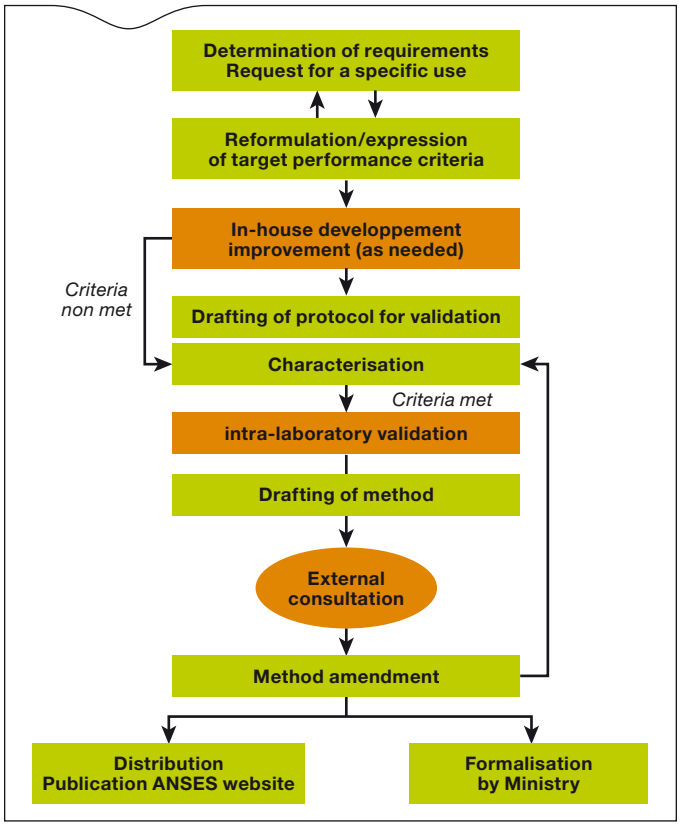

Figure 1. Process for preparing and formalising methods in plant health

the Plant Health Laboratory has drawn up an in-house guide on characterisation of method performance criteria.
Generally, methods used by the NRL or intended for formalisation undergo characterisation of the following technical performance criteria (see Definitions, Box 2):
- sensitivity (in terms of inclusivity), specificity and accuracy;
- detection limits;
- repeatability;
- intermediate precision (intra-laboratory reproducibility).

Other non-technical criteria such as cost, ease of use and so on, are evaluated on a case-by-case basis.
At the start of 2013, revision of this guide prompted the laboratory to:
- introduce a calculation for uncertainty regarding the sensitivity, specificity and accuracy parameters;
- make provisions for studies on robustness with minor but deliberate variations in parameters that are important for the overall reliability of results, for the specific case of methods that are to be delegated.

The full results of characterisation testing are compiled in a report. These data are then compared with the predetermined target performance criteria to decide on the degree to which the method meets its intended use.
- if the target criteria defined by the client cannot be fulfilled due to technical limitations:
  - work is carried out to optimise the method or develop a new method,
  - or the specifications are amended by the client,
  - or implementation of combined methods and/or restricted conditions in which the methods can be used;
- if the target performance criteria are met, the method:
  - can be validated if it is to be used in-house by the NRL;
  - is submitted for external consultation if it is to be delegated to a network of accredited laboratories.

Ultimately, this intra-laboratory characterisation of performance criteria is very similar to the process that may be followed in the other areas of expertise within ANSES, such as animal health or food safety. However, a moderate number of samples are generally tested (depending on the pest of interest) compared to other areas, due to the low number of available naturally-infected samples. This is particularly true for pests that cannot be cultivated or that are difficult to maintain in reference collections.

External consultation

In agreement with the Ministry of Agriculture, the Plant Health Laboratory has included an external consultation phase in the method validation process, including:
- scientific peer review;
- public consultation.

Peer review is carried out at least for all methods intended for delegation, but may also be extended to methods used by the NRL. It is generally conducted by two French-speaking experts in the corresponding field of study.
Public consultation involves publication of draft methods on the Agency's website (French only - http://www.anses.fr/fr/content/m%C3%A9thodes-danalyse-dans-le-domaine-de-la-sant%C3%A9-v%C3%A9g%C3%A9tale), possibly after amendment further to the peer review process. Consultation is usually open for a period of two months. The aim of this phase is to obtain comments from the public, at least from future users, to identify potential implementation issues concerning the draft method from a technical point of view, or to obtain information on how well the operating procedures are understood.
The comments received are then used to draw up a final version of the operating procedure that takes account of different approaches to facilitate implementation and transfer to laboratories other than the one that developed and characterised the method performance criteria.
Although the public consultation process for draft methods is directly based on existing administrative or standardisation procedures, it is unique and specific to plant health among the various sectors in France involved in development of official methods.

Formalisation

As mentioned above, a method can only become official if the DGAL, as the risk management authority, indicates in writing that the method is to be used for official analyses.
As a result, once the final version is drafted following public consultation, the NRL submits the method to the DGAL along with all the data used to obtain its validation, specifically the performance criteria. On the basis of the submitted data, the DGAL can then formalise the method, unless the background context has changed or there are additional specifications.
In the past, formalisation of methods required a notice to laboratory heads to be published in the Official Journal of the French Republic. Some official methods that have not yet been revised according to the current process have still not been

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amended in the Official Journal. Formalisation now involves publication of an administrative notice 'note de service' by the DGAL that are made available to users, and more widely to the public, in the Official Bulletin of the Ministry of Agriculture (French only - http://agriculture.gouv.fr/bulletin-officiel). These notices specify in particular the methods' conditions of use (import, surveillance, etc.).
The methods themselves, i.e. the technical operating procedures, are made available at no cost to accredited laboratories and to the general public via the ANSES website (French only - http://www.anses.fr/fr/content/m%C3%A9thodes-danalyse-dans-le-domaine-de-la-sant%C3%A9-v%C3%A9g%C3%A9tale).

Conclusion

The procedure for formalising analytical methods in France in the area of plant health has been developed gradually by the National Reference Laboratory and the risk management authority. These interactions between the sponsor and service provider have helped to develop a framework that covers both the needs of the DGAL in terms of reliability and standardisation of analytical test results, and the needs of the NRL in terms of determination of expectations and accreditation requirements (so-called recognised methods). This model includes phases that are currently different from those in other areas of expertise within ANSES, specifically dialogue with future users via public consultations, and constitutes an interesting alternative to the conventional standardisation process.

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Methods for characterising resistance to carbamates, pyrethroids and neonicotinoids in *Myzus Persicae*

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The peach-potato aphid is a pest of several plant species. The use of plant protection products to limit the spread of this aphid in various crops can result in the selection of resistant individuals. Modification of the protein targeted by the insecticide is one of the most easily detected resistance mechanisms using tests relying on molecular biology methods. This article presents three methods of detecting various mutations that cause resistance to three insecticides very commonly used against the aphid: carbamates, pyrethroids and neonicotinoids.

Introduction

The peach-potato aphid, *Myzus persicae*, is highly polyphagous as it is capable of colonising over 400 different wild and cultivated plant species (herbaceous plants, fruit trees, shrubs, etc.). This phytophagous insect causes direct damage through its feeding punctures that weaken the plant and deform the leaves. It also causes indirect damage that can be highly destructive, since through its punctures, it is a virus vector that can transmit over 120 plant viruses affecting various plants and trees (CMV: Cucumber Mosaic Virus, CaMV: Cauliflower Mosaic Virus, Plum Pox virus affecting stone-fruit trees). In addition, the excretion of honeydew by the aphids promotes the growth of a fungus (*Fumago salicina*) known as sooty mould that makes the affected organs unfit for sale. *M. persicae* is also characterised by a complex biological cycle, stemming from its ability to reproduce both sexually and asexually and the fact that this cycle varies depending on the colonised plant and geographic location.

By virtue of its characteristics (asexual and sexual reproduction, polyphagia, virus vector), *M. persicae* is a highly destructive pest for numerous agricultural crops. The use of insecticides is one way of controlling this pest. And yet the repeated application of active substances can lead to the development of resistance to these products. For example, in the *M. persicae* species, resistance to several classes of insecticides has been reported. Several resistance mechanisms have been described, affecting four classes of insecticides approved for use against this pest in France (organophosphorous compounds, pyrethroids, carbamates, neonicotinoids). Resistance to insecticides in *M. persicae* is linked to two main types of mechanisms:

- **metabolic resistance** induced by the duplication of a gene that leads to increased production of the corresponding protein. The overexpressed protein is an enzyme capable of breaking down one or more active substances. For example, in *M. persicae*, carboxylesterases (E4 and FE4) are involved in moderate resistance to a broad spectrum of insecticides (carbamates, pyrethroids, organophosphorous compounds) (Devonshire *et al.,* 1982) while cytochrome P450 (Puinean *et al.,* 2010) is involved only in moderate resistance to neonicotinoids;
- **so-called target resistance**, caused by modification of the protein targeted by the insecticide, has also been described. This resistance mechanism is generally responsible for a very sharp decline in insecticide efficacy. Three main classes of insecticides are affected by this type of resistance: pyrethroids, carbamates and more recently neonicotinoids.

Regarding carbamates, a mutation responsible for a high level of resistance has been identified on the encoding gene for acetylcholinesterase 2 (Nabeshima *et al.,* 2003), a target of this insecticide class. At protein level, this mutation, referred to as MACE for "Modified acetylcholinesterase", occurs due to substitution of a phenylalanine for a serine at amino acid 431 of acetylcholinesterase 2 (S431F).

For pyrethroids, three mutations can cause target resistance to this class in *M. persicae*. They affect the insecticide's target: the voltage-dependent sodium channel. These types of resistance are called kdr (knock down-resistance) and s-kdr (super kdr). At protein level, the kdr mutation involves substitution of a phenylalanine for a leucine at position 1014 of the protein (L1014F). So-called s-kdr mutations affect codon 918 and involve a methionine mutation. Several substitutions have been described, for example, methionine can be replaced by a threonine (M918T) (Martines-Torres *et al.,* 1999). This mutation is always found in association with the kdr mutation (L1014F). The second substitution, which has been described more recently, involves a methionine-to-leucine replacement (Fontaine *et al.,* 2011). It has thus far always been found in the absence of kdr.

For neonicotinoids, the mutation involves substitution of a threonine for an arginine at position 81 (R81T) of the β1 subunit of the nicotinic acetylcholine receptor, the target protein for this insecticide class (Bass *et al.,* 2011).

As part of surveillance plans developed by the French DGAL (Directorate General for Food), the Resistance to plant protection products unit (RPP) monitors the development and spread of resistance to plant protection products in crop pests. In this context, for *M. persicae*, various analysis tools have been developed to detect four of the mutations causing resistance to insecticides in this insect. One of these tools can simultaneously detect resistance to carbamates caused by the MACE mutation of acetylcholinesterase and resistance to pyrethroids linked to the kdr mutation. A second tool seeks to detect the M918L mutation affecting the sodium channel involved in high resistance to pyrethroids. The last method presented here is used to detect the R81T mutation, in the β1 subunit of the nicotinic acetylcholine receptor (nAChR), which causes resistance to neonicotinoids.

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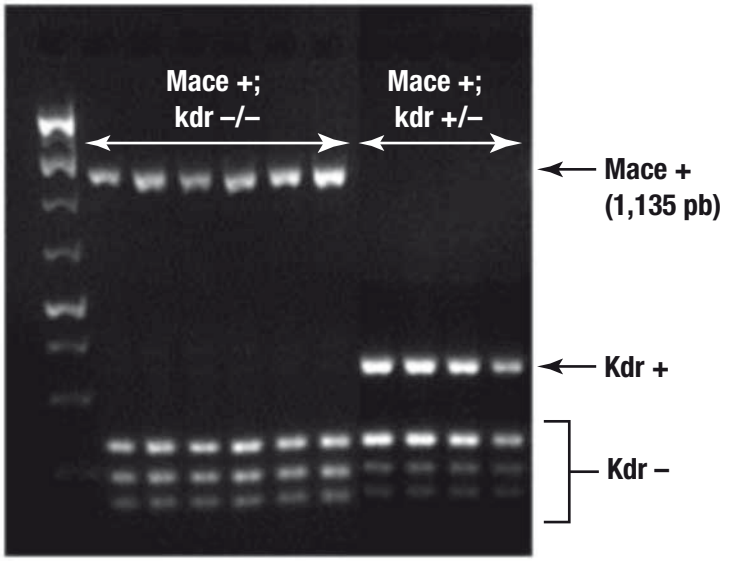
Methods for characterising resistance to carbamates, pyrethroids and neonicotinoids in Myzus persicae

Simultaneous detection of the modified acetylcholinesterase (MACE) and sodium channel kdr mutations involved respectively in resistance to carbamates and pyrethroids

This technique relies on multiplex PCR for amplifying a fragment of the sodium channel gene (para) containing the 1014 codon and amplifying a fragment of the acetylcholinesterase 2 gene (ace2) only when the S431F mutation occurs. Successful amplification of the para gene is a positive DNA extraction control in the absence of the mutated allele of the ace2 gene. For the kdr mutation, the presence or absence of mutation is then tested for by enzyme digestion, which can distinguish between the wild-type allele (two restriction sites, and three 159 pb, 193 pb and 256 pb fragments) and the mutated allele (one restriction site, and two 256 pb and 352 pb fragments) (Cassanelli *et al.,* 2005). Detection of the kdr allele indicates whether the aphid is heterozygous, homozygous susceptible or homozygous resistant (Figure 1). The method for detecting the MACE allele only indicates whether the allele is present or not and does not provide information about the individual's heterozygous or homozygous status. Since these two MACE and kdr resistance alleles are dominant, if they are found in the heterozygous state this therefore produces a phenotype with respective resistance to carbamates and pyrethroids.

Detection of M918L sodium channel mutations involved in resistance to pyrethroids

QPCR (quantitative PCR) with TaqMan probes is used for detecting the M918L mutation. The primers and probes were designed based on nucleic sequences and advice was kindly provided by Mr. Williamson (Rothamsted Research). The probe for detecting the wild-type allele (918M) is bound to fluorochrome Cy3 at the 5' end and BHQ2 at the 3' end. The probe for detecting the resistant allele (918L) is bound to fluorochrome FAM at the 5' end and BHQ1 at the 3' end. To increase specificity, each probe was designed with three LNAs (Locked Nucleic Acids). This technique can detect two alleles of the voltage-dependant sodium channel. It can determine whether the aphid is homozygous [MM], heterozygous [ML] or homozygous [LL] for codon 918. Following qPCR, after verification of the amplification curves, the probes' end-point fluorescence ratios are compared with one another (Figure 2) in order to define the genotype of each aphid.
Since the 918L resistance allele is dominant, if it is found in the heterozygous state it therefore produces a phenotype with resistance to pyrethroids.
The method presented here cannot detect the other mutation that can affect codon 918 (M918T). This mutation can be detected by a qPCR method (Anstead *et al.,* 2004). Multiplex testing with the three probes to simultaneously detect the three possible codons at position 918 (responsible for amino acids methionine, leucine and threonine) has not produced satisfactory results. Furthermore, other mutations affecting codon 918 have recently been highlighted (unpublished data).



PCR was performed with the Multiplex PCR Master Mix kit (Qiagen), 200 nM of each primer for detection of the S431F mutation in the ACE2 gene (AchE-F2 from Cassanelli *et al.* 2005 and Mace-R-Rev: TCAGCTGCCAATAATAAAATATAA) and 200 nM of each KDR-F1 and KDR-R4 primer (Cassanelli *et al.*, 2005) for amplification of the fragment in the *para* gene.

An initial denaturation phase at 94°C for 15 minutes was followed by 30 cycles at 94°C for 30 seconds, 60°C for 90 seconds and 72°C for 90 seconds. The 30 cycles were followed by a final extension of 5 minutes at 72°C.

Figure 1. Migration profiles, on a 2.5% agarose gel, of digestion products for the simultaneous detection of the modified acetylcholinesterase (MACE) and sodium channel kdr mutations.

Mace +; kdr –/–: *individuals with the MACE allele and without the mutated 'kdr' allele.*
Mace –; kdr +/–: *individuals without the MACE allele and heterozygous for the mutated 'kdr' allele.*

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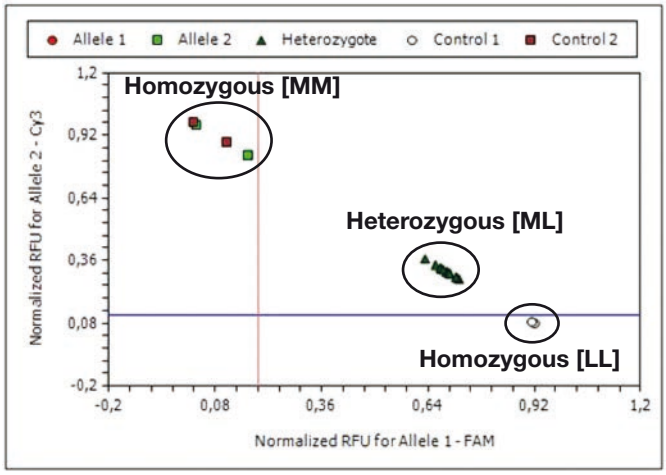

Primers and probes for TaqMan PCR, the nucleotides in brackets are Locked Nucleic Acids (LNA):

qMP SKDR-F:
GTGGCCCACACTGAATCTTTTAAT

qMP SKDR-R:
ACAAACGTTAGGTTACCCAAAGCA

Probe MPskdr-SBIS:
Cy3-ATGGTTCGACCC[+A][+T][+T]AT-[BHQ2]

Probe MPskdr-R- 918L:
FAM-ATGGTTCGACC[+A][+A][+T]AT-[BHQ1]

qPCR is performed in a final volume of 25 µL with 12.5 µL of Jumpstart Taqman (Sigma-Aldrich), 200 nM of each primer, 400 nM of each probe, 4.5 mM of MgCl2 and 1 µL of DNA. The PCR cycle consists of an initial denaturation phase of 2 minutes at 94°C and then 40 cycles with denaturation at 94°C for 15 seconds, annealing at 55°C for 30 seconds and then extension at 60°C for 45 seconds.

Figure 2. Result of aphid genotyping for codon 918 by TaqMan probe to detect the wild-type allele and the mutated 918L allele.
*The x-axis and y-axis show the fluorescence intensities of the two FAM and Cy3 fluorochromes used to distinguish between the three genotypes: **Homozygous [MM]:** aphid without a mutation at codon 918; **Heterozygous [ML]:** aphid having only one mutated allele at codon 918 (substitution of a leucine for methionine); **Homozygous [LL]:** aphid with the two mutated alleles at codon 918.*

Sense sequence for wild-type individual

5' - TAGTTCTAACTTATTGCCTGCAGCTATTAAAATATCCAATTAAATAATGT

GTCTTAATATTGTTTTATTGTTTAATGAAAAGAGTCAAATAATGAAATCAAAC

3' - AAAAGAGTCAAATAATGAAATCAAAC

***MPB1F-SmlI** sense primer* →

GTTTGGTTGAGACTTGTGAGTAACCTACTTAATATATATATTATATATATATA
GTTTGCTTGA – 5'

Base degenerated to create the restriction site

TTTATTTTCAGTTTGTAAACTATAAAATTAAAAAATAAACAGTTTCCTTTCTA

← ***MPB1TMR** anti-sense primer*

3' –ATAATCTGAAGGACTGGCG– 5'
ACGTATTAGACTTCCTGACCGC– 3'

Figure 3. dCAPS-PCR, position of the MPB1F-SmlI and MPB1TMR primers on the β1 subunit of the nicotinic acetylcholine receptor (nAChR) containing codon 81.
In red the mutated base for the dCAPS primer. In blue the base concerned by the R81T mutation.

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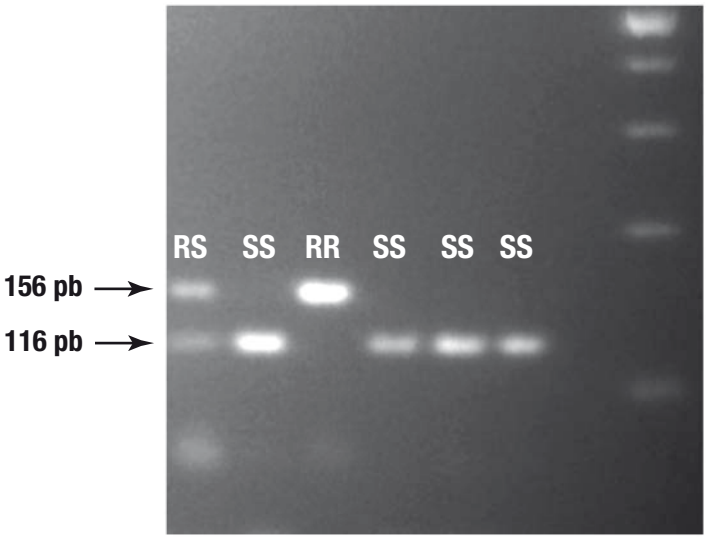

PCR is performed in a final volume of 25 µL with 200 µM of dNTPs, 300 nM of each primer, 2 mM of MgCl2, 0.1 µg/µl of BSA, 1.5 units of Taq and 1µL of DNA.

The PCR cycle consists of an initial denaturation phase of 5 minutes at 94°C and then 35 cycles including denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute and then extension at 72°C for 30 seconds. PCR ends with a final extension of 5 minutes at 72°C. Digestion of 10 µL of PCR products is performed with 5 SmlI enzyme units at 55°C overnight.

Figure 4. Migration profiles, on a 3% agarose gel, of the dCAPS-PCR digestion products, to detect the R81T mutation affecting the β1 subunit of the nicotinic acetylcholine receptor (nAChR).

Homozygous SS: *aphid without a mutation at codon 81;* ***Heterozygous RS:*** *aphid having only one mutated allele at codon 81 (substitution of a threonine for arginine);* ***Homozygous RR:*** *aphid with the two mutated alleles at codon 81.*

As a result, when analyses are undertaken with the two specific probes for the 918M and 918L alleles, for individuals with no fluorescence measurements or with ambiguous results, sequencing of a portion of the sodium channel gene containing codon 918 is performed in order to accurately determine the genotype.

Detection of R81T mutations in the β1 subunit of the nicotinic acetylcholine receptor (nAChR) involved in resistance to neonicotinoids

The method used is a dCAPS (Derived Cleaved Amplified Polymorphic Sequence) PCR. It relies on the creation of a restriction site when the allele is non-mutated. This site is created with a primer placed next to codon 81, one of whose bases, which is not complementary to the sequence for amplifying, results in the creation of a restriction site when the allele is non-mutated (Figure 3). Enzyme digestion can distinguish between the wild-type allele (116pb and 37pb) and the mutated allele lacking a restriction site (156pb) (Figure 4). In heterozygous individuals, the R81T mutation indicates decreased susceptibility to neonicotinoids but to a lesser extent than in homozygous individuals. This allele therefore appears co-dominant (unpublished data).

Conclusion

Myzus persicae is an ideal species for examining resistance to insecticides. Found on numerous crops, it is subject to various forms of phytosanitary pressure. Its biological cycle, during which sexual reproduction (allowing for genetic recombination) can alternate with asexual reproduction cycles (leading to rapid multiplication of the most advantageous genotypes), is an evolutionary advantage. The tools presented here allow for the targeted detection of some of the known resistance mechanisms in this aphid. They have been designed to meet the need to examine resistance alleles in *M. persicae* for three main classes of insecticides. However, the absence of a tested resistance allele does not necessarily indicate that an individual is susceptible to an insecticide. An aphid can have other resistance mechanisms not detected by any of the methods described here. Only insecticide susceptibility testing undertaken in a laboratory, by spraying or ingestion of an insecticide in controlled conditions, can exhaustively determine whether an aphid has a susceptible or resistant phenotype. Molecular tools, which are less burdensome to implement, can be used to determine the presence or absence of an allele recognised as causing resistance to one or more

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active substances. Their advantage lies in the fact that they can detect several mechanisms against different active substances, in the same individual. It should be noted however that considering the evolving capacities of this pest and changes in the use of insecticide classes, these analytical methods are likely to change or be replaced with new ones. For example, for resistance to pyrethroids, the development of an HRM (high-resolution melting) analytical method aiming to identify the various mutations affecting codon 918 of the para gene is currently being studied with a view to more exhaustive detection of the various alleles involved in resistance to pyrethroids.

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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

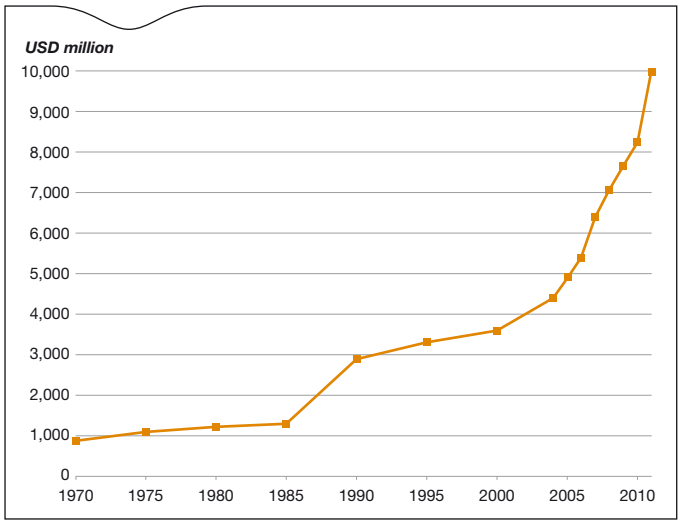

Figure 1. Trends in worldwide commercial seed trade (source: International Seed Federation)

Research for reference

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.

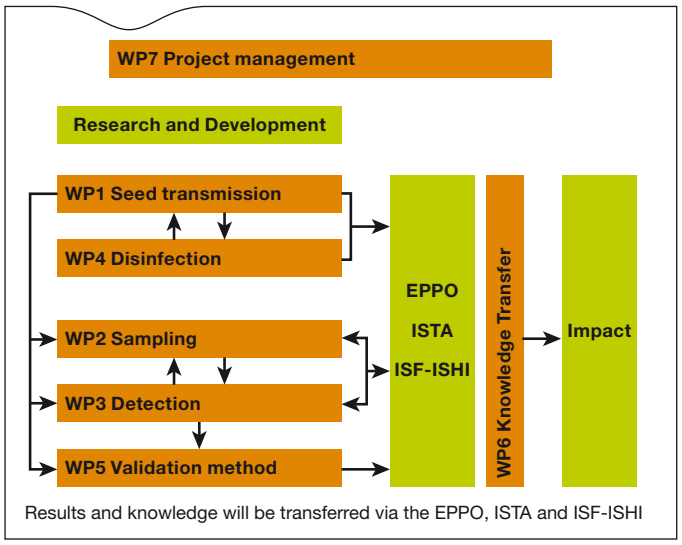

Figure 2. Organisation of the TESTA project.

Figure 3. Extracting the nematode *Ditylenchus dipsaci*

Research for reference

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.

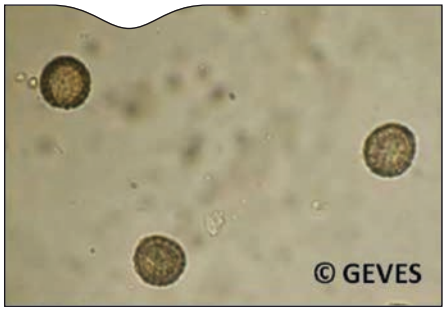

Figure 4. Teliospores of *Tilletia caries*

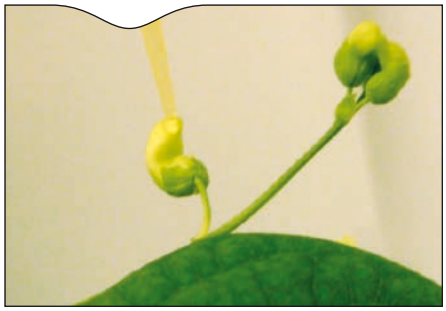

Figure 5. Artificial inoculation of flower buds

Figure 6. Healthy and contaminated lots of bean seed

Research for reference

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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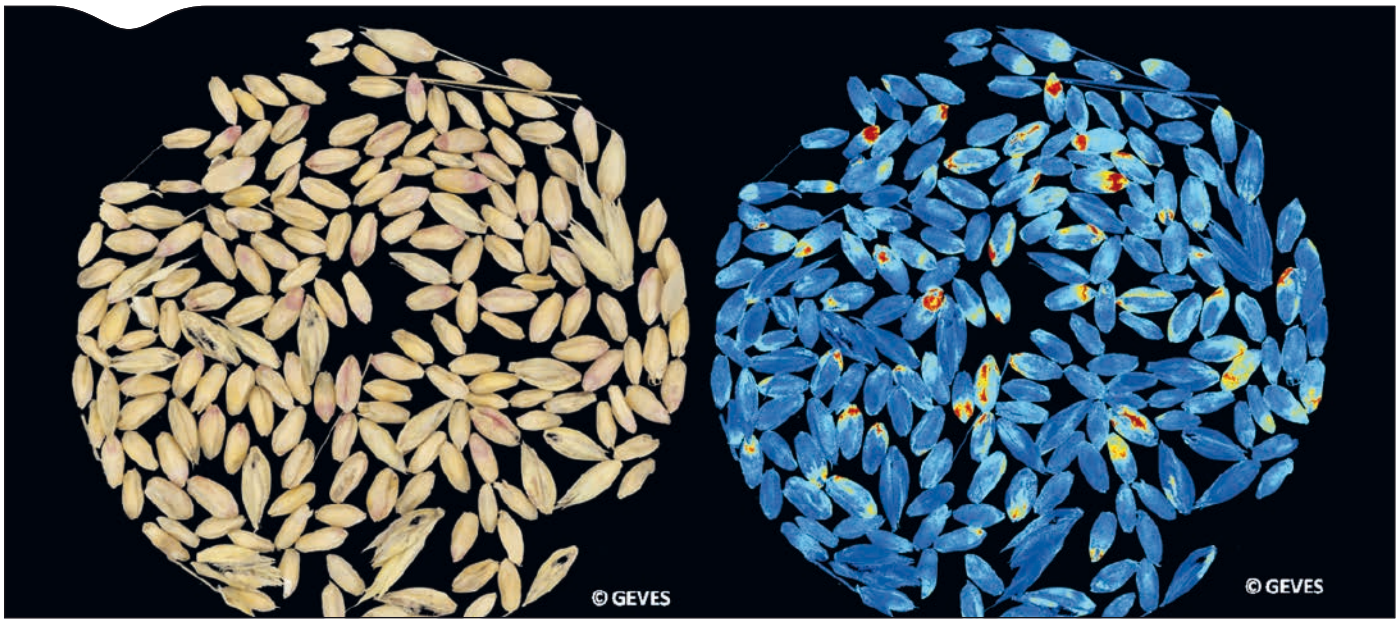

Figure 7. Detection of *Fusarium* sp. in wheat using a VideometerLab instrument.

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Networks

Organisation of Inter-Laboratory Proficiency Tests: feedback from the Plant Health Laboratory's Nematology Unit after almost 10 years

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This article describes the organisation of Inter-Laboratory Proficiency Tests (ILPTs) in the area of plant health through the example of ILPTs for the detection and identification of the potato cyst nematodes Globodera pallida (Stones) Behrens and G. rostochiensis (Wollenweber) Behrens. It first presents how participation in the network in France and Europe has changed, then describes the procedures for organising such tests. The main lessons learned by the organising unit are then given. Lastly, the issue of Inter-Laboratory Proficiency Tests in the area of plant health is broadened through other disciplines.

Introduction

In France, plant pests and particularly quarantine pests are monitored by a network of accredited laboratories in charge of undertaking official analyses for the French Ministry of Agriculture. One of the main missions of the National Reference Laboratory (NRL) is to ensure the reliability of such analyses by (i) overseeing this network, (ii) developing reliable and appropriate analytical methods in terms of performance for each intended use and (iii) organising Inter-Laboratory Proficiency Tests (ILPTs) requiring the participation of accredited laboratories and laboratories applying for accreditation. The primary aim of an ILPT is therefore to assess the participating laboratories by ensuring that they have the required capacities (proficiency) to conduct the analyses under their responsibility. As such, ILPTs rely on identical comparative media (test 'samples' or portions) for all of the participating laboratories and the results are compared with satisfaction criteria (success) that are established before the samples are sent out.

Furthermore, a two-pronged quality approach is used for the organisation of ILPTs and participation in these tests.

- Participation in ILPTs has historically been voluntary with the aim of helping French and European laboratories implement quality assurance. In France, participation is recommended by the guidance document of the French Accreditation Committee, LAB REF 02 (COFRAC, 2012), which aims to provide clients with high-quality, verifiable analytical activities.
- In addition, the quality approach implemented by the Plant Health Laboratory (LSV) is currently being strengthened so as to ultimately offer ILPTs developed in accordance with the ISO 17043 (ISO, 2010) Standard by 2015. This international standard defines general requirements for the competence of providers of proficiency testing schemes and for the

Globodera pallida and *G. rostochiensis*: two quarantine nematodes

These two nematodes are obligate parasites specific to solanaceous crops, especially potatoes. Native to the Andes and particularly Peru (Picard *et al.,* 2004), they have proliferated widely through the spread of potato crops and can now be found on all continents. Protected inside a cyst (Photo 1), a form of preservation for these parasites, the larvae (Photo 2) can survive in the soil for around ten years in the absence of a host plant (Wright and Perry, 2006). This form of preservation gives them a dispersive advantage particularly through trade, agricultural tools and other physical means of transport.

Severe reductions in yield (Greco *et al.,* 1982) linked to the damage caused to potato crops by these parasites justify their classification as quarantine pests under Directive 2000/29/EC of 8 May 2000 (Anonymous, 2000) and therefore the adoption of mandatory control measures. Specifically, this European regulation stipulates (i) that potato tubers (*Solanum tuberosum* L.) intended for planting must come from fields free from these two nematodes and (ii) that potato plants must be produced on uncontaminated land.

Therefore, compliance with the regulations in force necessarily implies the use of nematode extraction techniques based on samples of soil and underground plant parts, and species identification techniques. In France, the National Reference Laboratory is responsible for developing such methods, which are officially published and applied by accredited French laboratories.

Photo 1. Nematode cysts of the *Globodera* genus (Source, LNPV).

Photo 2. Anterior end of *Globodera* larva (Source, LNPV).

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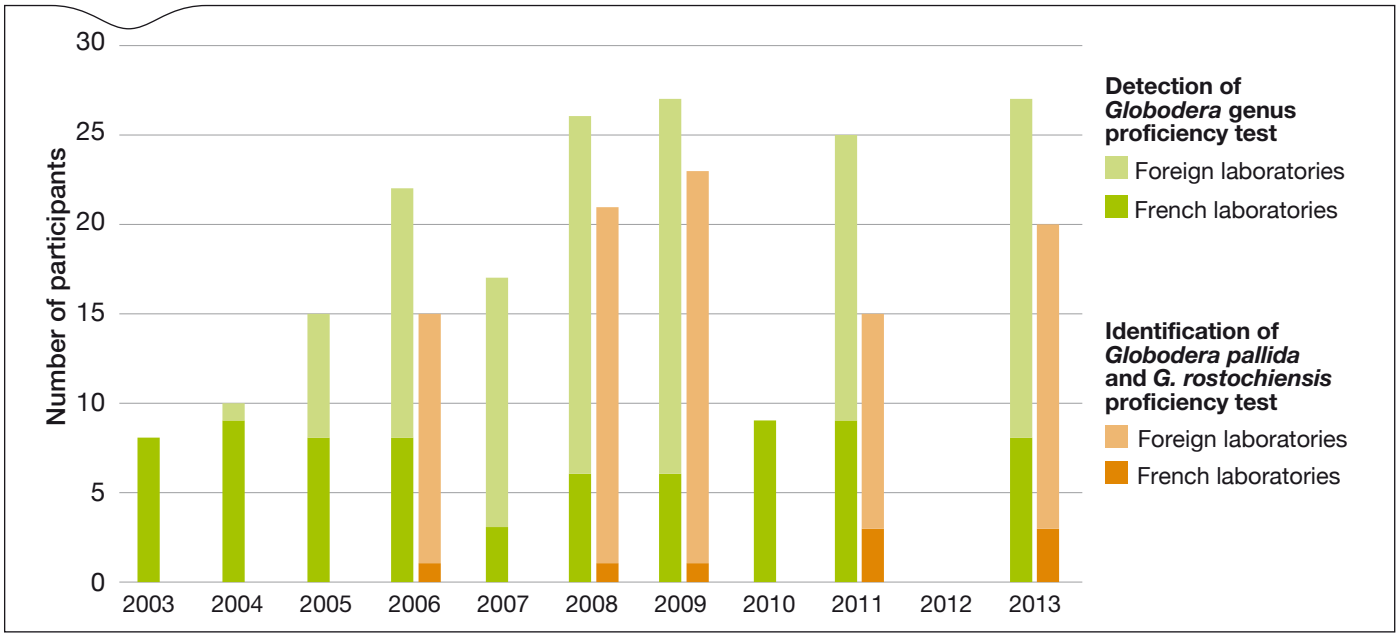

Figure 1. Changes in the number of French and foreign laboratories participating in ILPTs for the "Detection of *Globodera* genus nematodes" and the "Identification of the *G. pallida* and *G. rostochiensis* species".

development and operation of such schemes. This process helps improve the reliability of the official analyses undertaken by the network of accredited and reference laboratories.

The Nematology Unit, which is the NRL for plant-parasitic nematodes, has been organising ILPTs for the potato cyst nematodes *Globodera pallida* (Stones) Behrens and *G. rostochiensis* (Wollenweber) Behrens for almost ten years (Box 1). After an initial section describing changes in participation, the second part of the text will present the methodology used in 2013. The last part of the article will be devoted to feedback from the NRL regarding ILPTs.

Changes in participation in ILPTs dedicated to Globodera genus nematodes

The Nematology Unit has been organising ILPTs for the detection of the *Globodera* genus for ten years and for the identification of the *G. pallida* and *G. rostochiensis* species for five; there have been significant changes over this period.

In 2003, the first ILPT devoted to the detection of the Globodera genus was organised and was initially open exclusively to French laboratories. Then in 2004, certain European laboratories were invited to participate on a voluntary basis. The number of ILPT participants has since increased over the years.

From 2003 to 2006, the French participants were mainly French Regional Plant Protection Laboratories (LRPVs) and professional laboratories. After 2006, when the network of laboratories in France was restructured, giving rise to the gradual disappearance of these LRPVs, the number of participating French laboratories decreased while the proportion of foreign laboratories rapidly increased (Figure 1). Also in 2006, the first ILPT for the identification of the *G. pallida* and *G. rostochiensis* species was held and was almost exclusively intended for participants from other European countries (Figure 1). The panel of participating European laboratories is made up of National Reference Laboratories, regional laboratories and professional laboratories. As such, over the past ten years, a total of 54 laboratories(1) from 22 countries have participated in an ILPT session for the detection of *Globodera* genus nematodes (Figure 2), whereas 36 laboratories(1) from 20 different countries have participated in ILPTs for the identification of the *G. pallida* and *G. rostochiensis* species (Figure 2).

Organisation of ILPTs dedicated to *Globodera* genus nematodes

The key stages of the methodology used in 2013 are summarised chronologically in Table 1. The analysis stage is described in detail below.

Analytical methods

The analyses undertaken by French laboratories are described in the official methods for the "Detection of *Globodera* genus nematodes" (Anonymous, 2011) and the "Identification of *Globodera pallida* and *G. rostochiensis* by morpho-biometric and biomolecular analysis" (Anonymous, 2012). It should be noted that French participants, and particularly accredited laboratories and laboratories applying for accreditation, are required to strictly follow these methods. Foreign participants can use the method of their choice, generally the one that is routinely used in their laboratory. The requirements specific to each ILPT are described below:

- ILPT for the "Detection of the *Globodera* genus": regardless of the origin of the participating laboratories, soil samples undergo an extraction process usually using a Seinhorst elutriator or a Schuiling centrifuge (or any other similar device).

(1) Each participating laboratory has been counted only once for the entire period in question.

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Table 1. The main organisational stages for the 2013 "Detection of the *Globodera* genus" and "Identification of *Globodera pallida* and *G. rostochiensis*" ILPTs

Stage	Details
Call for applications	The participants are: • in France, laboratories accredited by the Ministry of Agriculture and laboratories applying for accreditation; • in Europe, National Reference Laboratories, regional and professional laboratories.
Preparation of comparative media	The panel subject to analysis is made of 10 samples identified by a unique code. Each sample contains: • for the "Detection of the *Globodera* genus" ILPT, soil that is free from or has been artificially infested with *Globodera* sp. cysts (several contamination levels); • for the "Identification of *Globodera pallida* and *G. rostochiensis*" ILPT, 3 isolated cysts.
Analyses (See § on Analytical methods)	The method used is: • for French laboratories, the official analysis method (MOA019); • for foreign laboratories, the method of their choice, which is generally the one they routinely use.
Test report: notification of compliance or non-compliance of results	Expected performance levels: • 100% sensitivity (all positive samples are found positive by the participating laboratory); • 100% specificity (all negative samples are found negative by the participating laboratory); • 100% accuracy (summary of the above two criteria).
Satisfaction survey	The results of the satisfaction survey are processed so as to continuously improve the organisation of ILPTs.

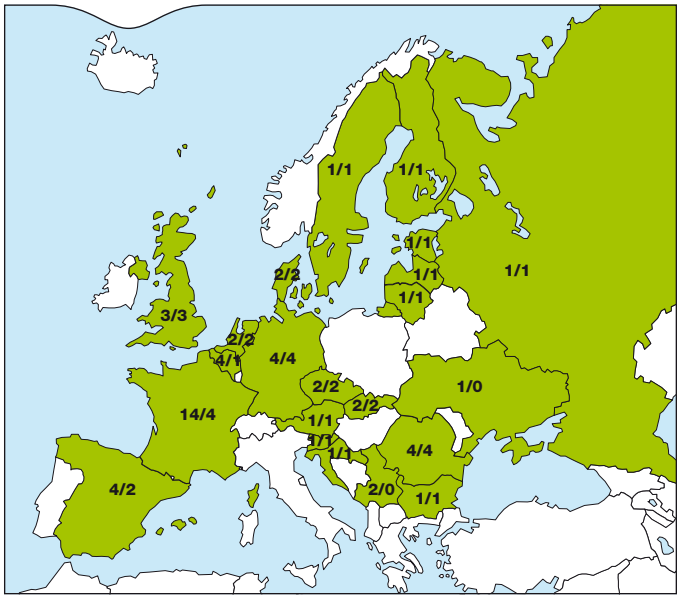

Figure 2. Distribution and number of laboratories participating in the "*Globodera*" ILPTs organised by the LSV's Nematology Unit.
Left-hand figure: total number of laboratories that participated in the ILPT for the "Detection of* Globodera *genus nematodes" from 2003 to 2013; right-hand figure: total number* of laboratories that participated in the ILPT for the "Identification of* G. pallida *and* G. rostochiensis *species" from 2006 to 2013.*

* Each participating laboratory has been counted only once for the entire period in question.

The extract is then examined with a stereomicroscope to screen for cysts without vulval cones. Morphological criteria are taken into consideration for this (presence or absence of vulval cones among other things) to identify the genus. Therefore, for each sample, the participating laboratories must decide whether or not there are cysts without vulval cones and then return the detected cysts to the organiser for confirmation of the genus.

- ILPT for the "Identification of *G. pallida* and *G. rostochiensis*": French laboratories, applying the official method, base their identification on morpho-biometric criteria applicable to cysts (Granek's ratio which is the distance from the vulva to the anus divided by the diameter of the vulva; number of cuticular ridges found between the vulva and the anus) and larvae (shape of stylet knobs and stylet size). This identification is supplemented by a molecular analysis (DNA amplification by conventional PCR) of the larvae. The results obtained with these two techniques are then compared and a conclusion on the final status of the sample is issued. Foreign laboratories can use the method of their choice (morpho-biometric and/or biomolecular). Like above, for each sample, the laboratories decide as to the presence or absence of *G. pallida* and/or *G. rostochiensis*.

Feedback on ILPTs dedicated to *Globodera* genus nematodes

The organiser's feedback

The organisation of ILPTs first requires the production and maintenance of biological reference materials. To that end, the Nematology Unit has been maintaining a collection of species and species populations since 2003. The growing number of participants in the two ILPTs has required the production of appropriate reference materials for the preparation of comparative media.

Networks

To ensure the conformity of these materials, the Nematology Unit has improved its verification processes over the years. Specifically for the ILPT for the "Detection of the Globodera genus", this involves conducting preliminary tests in the matrix (soil) to guarantee the absence of target organisms (cysts without vulval cones such as *Globodera* sp. and *Punctodera* sp.). Moreover, to prevent the risk of cross contamination, the preparation of samples at various levels of contamination is separated over time and/or space (preparation of healthy samples and then contaminated samples in dedicated areas of the laboratory). Furthermore, 20 samples per contamination level undergo homogeneity tests before the panels are sent to the participating laboratories. Stability tests are not necessary since *Globodera* sp. cysts can persist for several decades (Wright and Perry, 2006). Lastly, dual verifications, undertaken by different operators, are performed to ensure (i) each sample's compliance with the level of contamination and (ii) agreement between the reference (code) assigned to each sample and its status.

Other practical aspects can be raised, related to the development of procedures, specific documentation and data input. For example, each participating laboratory signs a participation contract setting forth each party's commitments, among other things. Starting in 2013, to limit errors in the reproduction of results, the organising laboratory will ask the participants to submit their results electronically, preferably on a form (Word document) sent by email.

An analysis of the results obtained through the various ILPT sessions for the "Detection of the *Globodera* genus" (Ladevèze and Anthoine, 2010) showed that the likelihood of detecting a cyst in a sample is not related to the total number of cysts occurring. Therefore, the likelihood of detecting at least one cyst in a sample containing n cysts is defined by the following formula: $P = 1-(1-p)n$. This relationship has been used to determine more appropriate levels of contamination above the limit of detection to be submitted to the participating laboratories.

Participant feedback

For French laboratories, the main objectives are to comply with (i) the official method in force when undertaking analyses and (ii) the deadlines for conducting analyses and submitting results. An analysis of the results obtained from the ILPT for the "Detection of the *Globodera* genus" showed that some participants mistook nematode cysts for other elements (seeds, propagules other than nematode cysts, etc.). Although this sorting error was then corrected in the identification stage, it shows that it is difficult to delegate methods based on morphological criteria. The ILPTs for the "Identification of *Globodera pallida* and *G. rostochiensis*" that have been organised since 2006 have confirmed the benefits of combining morpho-biometric and biomolecular analysis techniques rather than using just one analysis technique (biomolecular or morpho-biometric), to guarantee more reliable results (Ladevèze and Anthoine, 2010).

Conclusion and outlook

Like the Nematology Unit, all of the Plant Health Laboratory's units now organise Inter-Laboratory Proficiency Tests in their areas of expertise (see 2013 timetable given in this issue of *EuroReference*). This has occurred slightly more recently, with the delegation of official analyses to the network of accredited laboratories. Again, just like in the unit used as an example in this article, participation in these ILPTs is gradually opening up to European laboratories, particularly the reference laboratories of other European Union Member States and countries with which the laboratory collaborates. Documents are gradually being translated into English alongside the implementation of the quality approach in accordance with the ISO 17043 standard to allow these foreign countries to participate. These countries have responded extremely favourably to this type of proposal, as there are very few ILPT providers (or next to none for certain disciplines such as mycology and entomology) in the area of plant health in Europe.

It should however be noted that the participating laboratories must be authorised, by their official services, to receive samples containing quarantine pests.

While the laboratories participating under an accreditation for the French authorities are required to adhere to the official methods, in most cases, foreign participants can use the detection protocol of their choice for the proposed samples. These protocols rely on different approaches (morphometric, biomolecular, serological, etc.).

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Networks

ENGL, the European Network of GMO Laboratories

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When the current EU policy on Genetically Modified Organism (GMO) was designed, GMO-specific detection methods were not generally available. Under the leadership of the Joint Research Centre of the European Commission (JRC), a European Network of GMO Laboratories was formed, the ENGL.
ENGL brings together the GMO control laboratories (including national reference laboratories – NRL) of the EU to establish standards, spread good practices, and discuss problems. Its standards are today used throughout the EU and beyond.
ENGL shows how an EU-wide network supports the level playing field that is essential for un-disturbed trade, and how it benefits its members in their daily work.

Introduction and background to ENGL

The European Union authorises genetically modified organisms (GMO) for entering the market only after careful risk assessment by EFSA, the European Food Safety Authority. Respecting the consumer right-to-know, it is also mandatory to label any food or feed product that contains an ingredient of which more than 0.9% of its mass are from an authorised GMO and even if this presence is fortuitous or technically unavoidable.
The EU Regulation (EC) No 1829/2003 on genetically modified food and feed (http://gmo-crl.jrc.ec.europa.eu/legalbasis.htm) makes validated methods for detecting GM food and feed a pre-condition for authorisation and requires that these methods are equally applied throughout the EU.
When this policy was conceived, PCR-based methods for GMO detection just became available and were not yet commonly applied in the food and feed control laboratories in the EU-Member States. In order to support their introduction and their harmonised application, the European Network of GMO-Laboratories (ENGL) was officially set-up in 2002. Since then the network is instrumental for adapting the EU GMO control system to scientific progress.

ENGL - membership, structure, and function

All official GMO control laboratories of the EU Member States, including the NRLs for GMO, are members of ENGL. The ENGL is led by the European Union Reference Laboratory for GM Food and Feed (EU-RL GMFF, http://gmo-crl.jrc.ec.europa.eu/), hosted by the JRC. GMO laboratories from non-EU states are also participating as members (EEA) or observers. See the list of participants at: http://gmo-crl.jrc.ec.europa.eu/ENGL/ENGLmembers.htm
The EU-RL GMFF chairs the network and provides its secretariat. It organises and finances the meetings of the ENGL plenary, the ENGL Steering Committee and the ENGL working groups.
There are 2 ENGL plenary meetings per year, open also to observers. They normally take place in Ispra, north Italy, the seat of the EU-RL GMFF. Their main function is to update the network on new developments and allow for face-to-face networking of the members. They also serve for sharing good practices and discussing common problems, establishing the work plan of the network and exchanging ENGL documents approved by the ENGL Steering Committee.

The ENGL Steering Committee (ENGL-SC) consists primarily of the NRL for GMO. It also meets twice per year to prepare the ENGL - plenary meetings, to establish the ENGL work plan, to decide about the creation and mandate of ENGL working groups and monitor their progress, and to adopt their final results as ENGL products. Currently the ENGL has four active working groups:

WG MPR (Method Performance Requirements)

This WG works on the document "Definition of minimum performance requirements for analytical methods of GMO testing". The aim is to broaden the scope of the current document (http://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm) to qualitative, taxon-specific, DNA extraction and multiplex methods. Criteria for defining false positives, false negatives, and for assessing the performance of qualitative detection methods are discussed, as well as a protocol for testing methods' robustness and general criteria for DNA extraction methods.

WG SPP (Sample Preparation Procedure)

The WG pulls together good sampling practice and is preparing a guidance document on Sample Preparation Procedures. The final draft is at an advanced stage and will inter alia advocate performance tests for the different steps of the sample preparation. Good working procedures will be described.

AG SMV (Advisory Group on Selection of Methods for Validation)

The mandate of this advisory group is, opposite to the WGs, not limited in time. The group identifies detection methods that should be validated for filling gaps in the regulatory GMO detection toolbox. So far taxon-, element-, and construct-specific methods have been suggested by the ENGL members and a priority list will be proposed by the group to the ENGL-SC for adoption. Thereafter the ENGL members will be invited to contribute and participate in their validation, which will be led by the EU-RL GMFF.

WG DIR (Detection Interpretation Reporting)

This WG shall produce / update practical guidance for the detection, identification and quantification of GMOs in food or feed, the interpretation of analytical results, and their reporting.

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It will address authorised GMOs, for which event-specific methods are available, and unauthorised GMOs, for which those methods are normally lacking. To embrace the broad scope of the document, activities were divided in three sub-groups:

- G1 - Cut-off values and verification of analytical results,
- G2 - Matrix approaches and reporting,
- G3 - Knowledge-based approaches and new technological developments.

ENGL results

After more than ten years of existence, the importance of ENGL for the harmonisation of GMO detection throughout the EU and beyond cannot be overestimated. Based on its vast hands-on expertise it ensured that its generally accepted method performance criteria, against which the performance of methods can be assessed, are realistic and feasible for practical control situations. By bringing together the entire regulatory GMO analysis expertise of the EU it is highly respected as authority in this field.

The ENGL has also produced a number of guidance documents that are published on the EU-RL GMFF website and therefore globally available (http://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm). Certain of them became a global de facto standard and the ENGL itself serves as example for other regions of the world where similar networks are forming. ENGL members are regularly called upon to provide training on GMO analysis to colleagues from third countries.

Generally speaking, ENGL organisation, practices and documents also served as models in other detection areas such as food microbiology.

ENGL outlook

The number of GMOs (plants and animals) that are reaching and will reach commercialisation is increasing. Their regulatory management will continue to need (cost-) effective, reliable methods for their detection, identification and quantification. ENGL's expertise will remain instrumental for the development and global acceptance of such practically feasible methods, even if the challenges resulting from new breeding techniques and the possible introduction of GM-animals into the food and feed chain should not be underestimated.

These big challenges that lie ahead result from scientific and technological progress. New techniques of genetic engineering challenge current detection methods and require that new analytical techniques must be implemented by the community of the EU GMO-control laboratories. The ENGL will support this implementation while continuing ensuring that proposed methods remain feasible, both in terms of scientific and technical complexity and cost.

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Q-bacco-net: An initiative to ensure availability of high quality reference material of plant quarantine bacteria in support of research and European plant protection

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Reliable diagnosis and detection of quarantine bacteria are crucial for European agriculture. The establishment of efficient diagnostic and detection methods is reliant on expertise and well characterised reference material which is representative of the considered taxa and includes related strains as well as non-relatives which share diagnostic features (so called "look-alike" strains). To improve access to these resources, three public collections, BCCM/LMG, NCPPB and CIRM-CFBP have associated as the Q-bacco-net initiative, stimulated by ILVO and supported by EPPO. This network aims to underpin research and diagnosis of quarantine bacteria by proposing a panel of relevant reference strains for each quarantine pathogen.

International trade and travel has increased tremendously in recent years with plants and plant products being moved into and from the European Union. As a consequence, the rate of introduction and establishment of new, economically or environmentally damaging plant organisms and invasive species has increased steadily. Climate change may also increase the probability of establishment of organisms in areas other than their area of origin. Such organisms include plant-pathogenic bacteria.

Twenty-seven taxa of bacteria are currently identified as posing an unacceptable risk to agricultural and horticultural crops, forest and the wider environment and have consequently been included in the Annexes of the Directive 2000/29/EC as pests of the European Union (these lists of organisms are also called quarantine pest lists). The lists of pests recommended for regulation and of pests representing a putative risk to the European and Mediterranean region are available through the EPPO (European Plant Protection Organisation) website respectively as A1/A2 Lists of Pests and the Alert List (http://www.eppo.int/QUARANTINE/quarantine.htm).

In the absence of any curative methods for bacterial plant diseases, the only options for control remain avoidance, prevention and prophylaxis. As a consequence, detection of quarantine bacteria is of utmost importance in the adoption of relevant measures to prevent their dissemination in support of European plant protection. Detection and identification of quarantine bacteria must be reliable since the consequences of mis-detection (false negative or false positive findings) can be dramatic, from both economical and agricultural points of view. In order to design reliable detection/diagnostic methods, it is necessary to have access to reliable, well-characterised reference material. Ideally, this material should offer an overview of the considered taxa with respect to diversity and diagnostic characteristics. Control strains should be representative of the known diversity within the taxa. Negative control strains should include closely related strains as well as non-relatives, which share diagnostic features (so called "look-alike" strains), and which can be isolated simultaneously with target quarantine bacteria.

Three well-established public collections of plant pathogenic bacteria (BCCM/LMG, CIRM-CFBP and NCPPB), operating under ISO 9001:2008 certification, have been associated within Q-bacco-net, a new initiative stimulated by the Institute for Agricultural and Fisheries Research (ILVO) and supported by the Dutch Q-Bank project and the European Plant Protection Organisation (EPPO). These collections have agreed common panels of reference strains (Q-bacco-ref) for each bacterial taxon listed by EPPO as A1 or A2 pests or on the Alert List. The reference strains were selected using the following criteria:

- they represent the complete known diversity of considered taxa and also include closely related and look-alike strains;
- they are well characterised phenotypically and genetically;
- they cover the range of geographical and biological origins;
- they include, where relevant, species or subspecies type strains, pathovar reference strains and whole genome sequenced strains.

Common quality standards are applied to their characterisation, authentication, maintenance, storage and distribution. The reference panels are intended for display through the EPPO website. More information about these strains is available on the websites of the respective collections and also through StrainInfo (http://www.straininfo.net/).

The three collections decided to share their biological resources to ensure the strains of the panels are available in at least 2 of the 3 collections, to ensure their continued accessibility and availability to the community of diagnosticians and scientists, including the national plant protection organisations. These public collections are well placed to propose these reference strains through their existing strategic collaboration with research specialists in plant-pathogenic bacteria, through their core mission of preserving biological resources and associated data, and of organising access to these resources in full compliance with (inter)national legislation (Janssens *et al.,* 2010).

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It is expected that the reference panels will evolve with time, to stay in line with known diversity and taxonomic descriptions of target organisms and their look-alikes, and to take into account new additions to the target lists. Arrays of genetic markers for each target organism are also expected to increase. With Q-bacco-net, the three public collections aim to underpin the activities of diagnostic and research labs by improving access to well-characterised reference strains, and to facilitate new developments of reliable and effective detection/diagnostic methods as well as assisting in their validation and ensuring that they are used proficiently.

Reference

Janssens, D., D. R. Arahal, *et al.* (2010). «The role of public biological resource centers in providing a basic infrastructure for microbial research.» Res Microbiol161(6): 422-429.

Agenda

Interlaboratory comparisons organised by laboratories in the EPPO region

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Since 1998, the European and Mediterranean Plant Protection Organization (EPPO) has established a work programme in the area of plant health diagnostics to harmonize procedures across the region. The work is conducted by the Panel on Diagnostics and Quality Assurance in collaboration with specialized Panels (Diagnostics in Bacteriology, Entomology, Nematology, Virology and Phytoplasmology and the European Mycological Network). The EPPO Panel on Diagnostics and Quality Assurance is preparing a Standard to provide guidance for the organization of interlaboratory comparisons by plant pest diagnostic laboratories.

In order to make further progress on the draft standard, it was considered important to gather information on the procedures followed by laboratories organizing proficiency testing and test performance studies (frequently called ring tests). An online survey was organised between September 2012 and January 2013.
In total, 52 laboratories from 28 countries answered the survey. Laboratories were asked if they had already organised proficiency testing and/or test performance studies and on which pest/matrix combination. The list of laboratories and test/matrix combination concerned are presented in the tables below.

Pest/matrix combination for proficiency testing

Pays	Laboratory name	Pest/matrix combination
France	ANSES, Plant Health Laboratory	• *Chalara fraxinea*/*Fraxinus* spp. • *Phytophthora ramorum*/*Rhododendron* spp. • *Monilia fructicola*/*Prunus persica* • *Gibberella circinata*/Seeds of *Pinus* spp. • *Gibberella circinata*/Pure culture • *Plasmopara hasltedii*/Seeds of *Helianthus annuus* • *Ceratocystis platani*/*Platanus* spp.
		• Viruses/leaves of *Musa* spp.
		• *Globodera pallida* and *Globodera rostochiensis*/Soil • Female of *Meloidogyne* sp/*Solanum tuberosum* • *Ditylenchus dipsaci* and *Ditylenchus destructor*/Seed • *Bursaphelenchus xylophilus*/*Pinus* spp. wood extract
		• *Ralstonia solanacearum* and *Clavibacter michiganensis* subsp *sepedonicus* on potaoes
		• Beet necrotic yellow vein virus (BNYVV) on roots of sugar beet (*Beta vulgaris*).
		• *Bemisia tabaci* (puparium) • *Diabrotica virgifera* (adults)
		• Flavescence dorée and Bois noir phytoplasmas on *Vitis vinifera*
Germany	Analyse und Diagnoselabor im DLR Rheinpfalz	• Grapevine fanleaf virus (GFLV), Arabic mosaic virus ArMV); Grapevine leafroll-associated virus 1 (GLRaV-1, Grapevine leafroll-associated virus 3 (GLRaV-3), Grapevine fleck virus (GFIV) • Prune dwarf virus (PDV), Prunus necrotic ringspot virus (PNRSV), Plum pox virus (PPV)
	JKI-KLM, bacteriology	• *Clavibacter michiganensis* ssp. *sepedonicus*/*Solanum tuberosum* tuber extract • *Ralstonia solanacearum*/*Solanum tuberosum* tuber extract
Russia	Russian Plant Quarantine Centre	• Bacterial suspension (*E. amylovora*, *R. solanacearum*, *Clavibacter michiganensis* ssp. *sepedonicus*, *P. stewartii*)

Agenda

Pest/matrix combination for proficiency testing (cont'd.)

Pays	Laboratory name	Pest/matrix combination
Poland	Central Laboratory of the Main Inspectorate of Plant Health and Seed Inspection	• *Globodera* spp. – soil, cysts, DNA • Bursaphelenhus xylophilus/ specimens, PPV, PSTVd/lyophilized or fresh material • CMS/slides, *Diabrotica virgifera*/specimens, *Frankliniella occidentalis*/specimens • *Synchytrium endobioticum*/soil
Finland	Finnish Food Safety Authority Evira	• Tospoviruses INSV and TSWV in ornamental plants.
Belgium	ILVO - Unit Plant Sciences - Crop Protection	• *Phytophthora ramorum* and *Phytophthora kernoviae* on *Rhododendron* spp., *Viburnum* spp. and *Camellia* spp. • *Clavbacter michiganensis* ssp. *sepedonicus*/*Solanum tuberosum* tuber extract • *Ralstonia solanacearum*/*Solanum tuberosum* tuber extract • *Erwinia amylovora*/Extract from woody plants
Spain	Instituto Agroforestal Mediterraneo-Universitat politecnica de Valencia	• *Gibberella circinata* as pure cultures
	Laboratori de Sanitat Vegetal-Generalitat de Catalunya	• PepMV, TSWV, ToMV, TYLCV in *Solanaceae*
	Laboratorio regional de la C.A.R.	*Vitis vinifera* viruses (GFLV, GLRaV1, GLRaV3, GFKV AND ArMV): • *Vitis vinifera* fresh plant material; • *Vitis vinifera* extracts
Pays-Bas	Naktuinbouw Laboratories	• Leaves, seeds different pests
Slovénia	National Institute of Biology	• DNA isolated from mixture of *Ralstonia solanacearum* in potato extract • Immunofluorescence slides of *R. solanacearum* in *Solanum tuberosum* extract

Agenda

Pest/matrix combination for test performance studies

Pays	Laboratory name	Pest/matrix combination
France	ANSES, Plant Health Laboratory	• *Chalara fraxinea*/*Fraxinus* spp. • *Phytophthora ramorum*/*Rhododendron* spp. • *Monilia fructicola*/*Prunus persica* • *Gibberella circinata*/Seeds of *Pinus* spp. • *Gibberella circinata*/Pure culture • *Plasmopara hasltedii*/Seeds of *Helianthus annuus* • *Ceratocystis platani*/*Platanus* spp.
		• Bacteria (*Xanthomonas axonopodis* pv. *dieffenbachiae*)/Anthurium
		• *Meloidogyne chitwoodi* and *M. fallax*/DNA from soil extract • *Bursaphelenchus xylophilus*/DNA from *Pinus* spp. wood extract
Poland	Central Laboratory of the Main Inspectorate of Plant Health and Seed Inspection	• *Globodera* spp. – soil, cysts, DNA • *Diabrotica virgifera*/specimens • *Bursaphelenhus xylophilus*/specimens
Italy	CRA - Plant Pathology Research Centre	• Viruses: Plum pox virus on symptomatic and asymtomatic *Prunus* spp. leaves • Pepino mosaic virus on Solanum lycopersicum leaves, fruits and seeds, Tomato infectious chlorosis virus and Tomato chlorosis virus on Solanum lycopersicum leaves • Potato spindle tuber viroid on solanaceous ornamentals leaves • Grapevine viruses on *Vitis vinifera* bark • Phytoplasmas: '*Candidatus P. mali*' on *Malus domestica* apple leaves, '*Candidatus P. prunorum*' on *Prunus* spp. leaves • Bacteria: *Erwinia amilovora* on *Pyrus* spp. symptomless twigs, *Clavibacter michiganensis* subsp. *michiganensis* on *Solanum lycopersicum* seeds, *Xanthomonas arboricola* pv. *pruni* on symptomless *Prunus domestica* and *Prunus persica* different material, *Pseudomonas syringae* pv. *actinidiae* on pollen, symptomatic leaves and bark of *Actinidia chinensis* • Fungi: Monilinia fructicola on *Prunus persica*, *Tilletia indica* on teliospores, *Gibberella circinata* on *Pinus nigra* seeds, *Phytophtora ramorum* on fungal DNA
Belgium	ILVO - Unit Plant Sciences - Crop Protection	• Adults *Diabrotica virgifera* on pheromone traps
Netherlands	Naktuinbouw Laboratories	• Leaves, seeds different pests
	National Plant Protection Organization	• Various - focus on molecular biological detection and identification methods (conventional (RT) PCR (RFLP), real-time (RT) PCR, DNA barcoding).
Slovenia	National Institute of Biology	• DNA isolated from defined mixtures of *Erwinia amylovora* in host plant tissues (real-time PCR test)

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