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PLANT AND FOOD BIOSECURITY

Reporting

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PLANTFOODSEC

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Final Report Summary - PLANTFOODSEC (PLANT AND FOOD BIOSECURITY)

Executive Summary:
The European Union (EU) Networks of Excellence in Security project “Plant and Food Security” (PLANTFOODSEC) addressed biological threats to crops and food from production, through processing and marketing, to consumption. Unlike other EU initiatives in support of agricultural systems, PLANTFOODSEC focused on enhancing capabilities for prevention, detection, response, and recovery should crops or food be targeted with acts of bioterrorism or biocrime.
The project covered threatening plant pathogens and plant diseases (plant biosecurity) and also human pathogens (food biosecurity/food safety), primarily enteropathogenic strains of Escherichia coli and Salmonella spp., that can colonize and contaminate plants and food products at any point along the production and distribution chains, creating the risk of outbreaks of foodborne illness.
Funded by the European Commission’s Programme Security, the project includes the following areas of emphasis: epidemiology and crop biosecurity; food biosecurity; analysis of risks to European food systems and society from the intentional introduction of new pest and disease agents; development and deployment of diagnostic and detection systems; responder systems for eradication and containment; Training for plant and food biosecurity; dissemination, awareness and communication.
Funded for five years, the Network of Excellence renewed and reinforced a previously established European partnership on crop biosecurity to develop the capability and capacity to prevent, respond and recover from a biological incident or deliberate criminal act threatening the European agri-food system.
The ultimate goal of the project was to create a virtual research network (a virtual CENTRE OF COMPETENCE IN PLANT AND FOOD BIOSECURITY) in order to improve the quality and impact of training and research in relation to crop and food biosecurity in Europe, thus enhancing preparedness to prevent, respond to and recover from the natural or intentional introduction, establishment and spread of plant pests, pathogens and noxious weeds against crops in the European agro-food system.
The considerable amount of research promoted by the European Union – which has also involved non-EU countries, such as the United States, Israel and Turkey – brings to the development of a comprehensive set of tools for project end-users, i.e. the stakeholders of the agri-food chain, including producers, researchers, and the authorities responsible for plant health and food security.
Besides them, other project results include regulatory analysis and challenges identification, experimental and modelling approaches applied to plant disease epidemiology, molecular approach for diagnostics and

– more generally - the outcomes of training, dissemination and networking activities aiming to increase awareness on plant biosecurity and food safety among agronomists and food producers and within the scientific, policy and inspection areas.

Project Context and Objectives:
Agricultural crops are vulnerable to attack by a wide spectrum of insects and plant pathogens. The deliberate introduction of a new plant pest or pathogen into an agricultural area could have a serious impact on crop yield and on the cost of management over the short and long-term. Introduction of new pests and pathogens can disrupt the trade from a country or region, resulting in lost markets. Moreover, it may impose negative attributes on the quality of food and feed and may lead to unavailability of certain foods.
The European Union (EU) Networks of Excellence in Security project “Plant and Food Security” (PLANTFOODSEC) addressed biological threats to crops and food from production, through processing and marketing, to consumption. Unlike other EU initiatives in support of agricultural systems, PLANTFOODSEC focused on enhancing capabilities for prevention, detection, response, and recovery

should crops or food be targeted with acts of bioterrorism or biocrime.
The project covered threatening plant pathogens and plant diseases (PLANT BIOSECURITY) and also human pathogens (FOOD BIOSECURITY/FOOD SAFETY), primarily enteropathogenic strains of Escherichia coli and Salmonella spp., that can colonize and contaminate plants and food products at any point along the production and distribution chains, creating the risk of outbreaks of foodborne illness.
The term BIOSECURITY refers to protection from harm caused by biological agents. It covers food safety, zoonoses, the introduction of animal and plant diseases and pests, the introduction and release of living modified organisms and the introduction and management of invasive alien species. Biosecurity thus encompasses food safety, animal life and health, and plant life and health, including associated environmental risks. Interest in biosecurity has risen considerably over the last decade in parallel with the increasing trade in food and plant and animal products.
In fact, the globalization of markets, science and social links faces new challenges for plant health and food safety and security. Several plant pests are perceived as a threat to agricultural biosecurity and to agricultural industries in both developing and advanced industrialized countries.
PLANT BIOSECURITY has been defined as the protection of natural and managed plant systems from the emergence/introduction of pests that would negatively affect the productivity, sustainability or diversity of plant systems. PLANT HEALTH is of global importance for sustainable agriculture, food security and environmental protection.
Plant pathogens are globally distributed. However, the source of most plant pathogens, especially the crop-specific ones, is in most cases linked with the geographic origin of the host plant. At the crop origin, many highly contagious disease agents are endemic as part of an ecological equilibrium with the host plant and the biological environment. The global movement of crops (especially, staple and food crops) outside their natural environment enhances their vulnerability to pathogens at their new habitats, and might result in very significant consequences.
Outbreaks of plant diseases have significant negative impacts on global food security and they have been associated throughout the history, with crops outside their origin, resulting in a wide-scale famine. In 1845, for example, late blight of potato swept through Ireland, ruining the country‘s staple crop and in combination with other factors brought about the famine of 1845-46. The social impact of this disease was enormous, causing approximately one million deaths and forcing the emigration of a million others. Brown spot disease of rice contributed to the Bengal famine in India in 1942-43, in which nearly two million people died.
The significance of a disease outbreak within only one region can be significant even on a global perspective, if that region is a major world supplier for one of the food staples. For example, Fusarium head blight of wheat and barley has affected several successive harvests in several states of the USA between 1993 and 1998. In world agriculture, introduced pests have been estimated to cause annual losses of between USD 55 billion and USD 248 billion.
The International Plant Protection Convention (IPPC) has in its charge the global harmonization of phytosanitary measures set up by the different national plant protection organizations to prevent accidental introductions of exotic pests. Regional plant protection organizations, such as the European and Mediterranean Plant Protection Organisation (EPPO), aim at improving the harmonization of quarantine protocols. Today approximately 300 pests have been identified as quarantine pests, largely on the basis of EPPO's recommendations.
While most exotic invasive plant pests have been either accidentally introduced or passively spread for example by wind currents, little attention has been paid until recently to the possible, deliberate misuse of

plant pathogens as 'weapons' against agroecosystems.

Until recently outbreaks of foodborne illnesses caused by contamination of fresh produce with human pathogens such as E. coli O157:H7 and Salmonella spp. were reported in Europe relatively infrequently, and were generally of limited significance and confined to local areas. In 2011an outbreak of unprecedented impact of serious, in some cases lethal, enterohemorrhagic illness struck Germany and France, with smaller case numbers reported in other countries. Human health impacts due to the consumption of contaminated plant foods are not limited to cases associated with enteric human pathogens, however. Some fungal plant pathogens produce mycotoxins that are highly toxic to humans. Despite the potential damage that can result from consumption of aflatoxins, fumonisins, and other dangerous mycotoxins, our knowledge of these compounds in food products is limited, and there are few regulations addressing minimal allowable limits of such toxins in food.

Traditional thinking and planning regarding BIOTERRORISM has focused primarily on humans as the primary target. If the perpetrator’s objectives can be met solely through the creation of human illness, death and associated panic, this may be appropriate. However, if economic and political vulnerabilities are included as contributing factors, agricultural bioterrorism must be considered among other possible avenues of attack. AGRICULTURAL BIOTERRORISM (AGROTERRORISM) is a subset of bioterrorism, and it is defined as the deliberate introduction of an animal or plant disease/pest with the goal of generating fear, causing economic losses, and/or undermining environmental, social and economic stability. Agroterrorism in its widest meaning, including biological warfare, bioterrorism, biocrime and sabotage, is defined as the deliberate and malevolent use of pathogens by an individual, organization or State in order to damage the health of plants or animals, or even affect the use able to be made of them in terms of production, marketing, processing or consumption.

The food production system throughout the European Union, which includes farm production, harvesting, transport, processing, storage, marketing and consumption, is vast, complex and open.

The potential for terrorist attacks or other criminal actions against agricultural targets is increasingly recognised as a threat to international security. In the meantime, natural outbreaks of diseases demonstrate the destructive potential of an agroterrorism attack. Agriculture and related sectors upstream and downstream are essential to the social, economic and political stability of all nations.

Plant and food biosecurity is a relatively new field of research in Europe. The majority of work has been done in the United States, where many agencies and entities within the US government, State and local government, the private sector and universities are actively engaged in protecting U.S. agricultural resources from intentional or unintentional introduction of pathogens. Countries such as Australia and New Zealand also have invested in biosecurity research, notably on how to successfully exclude, eradicate and control unwanted agents that threaten the economy, environment and human health.

PLANTFOODSEC aimed to build a VIRTUAL CENTRE OF COMPETENCE in order to develop awareness and enhance EU preparedness in the event of the deliberate or accidental introduction of the most threatening organisms harmful to plants, thus enhancing preparedness and response capabilities to prevent, to respond and to recover from a possible use of plant pathogens against crops in the European agrifood system.

Following the recommendations of the Commission‘s European Security Research Advisory Board (ESRAB) the S&T objectives of the project referred to the following functional groups of capability to improve the EU bio-preparedness (to prevent, to recover and to respond) in the agrifood sector:

• Risk assessment, modelling and impact (analytical capacities)
• Training

• Detection, Identification and Authentication (surveillance capacities – detection systems)
• Situation awareness and assessment (biosecurity and bio-safety guidelines, professional code
• of conduct vs. dual use risks)
• Information management
• Intervention and neutralisation
In particular the PLANTFOODSEC aimed to achieve the following objectives:
• to develop knowledge frameworks and appraisal tools to counteract and respond to the possibility of introduction, outbreak and spread of the most threatening plant pests;
• to identify priorities for research and regulatory policy, and provide a baseline assessment of forensic capability to trace mycotoxins and human pathogens on plants (HPOP) enhancing the prevention, recognition, response, and recovery from foodborne illness due to the contamination of fresh produce;
• to develop models of spatial-temporal risk, to improve planning of effective and efficient national and regional responses;
• to improve disease surveillance and detection systems by facilitating international laboratory cooperation and by developing diagnostic tools;
• to prevent the establishment and spread within EU countries of deliberately-introduced pathogens by delineating the steps and the course of measures to be executed at European level in each category of pest detection and by enhancing the available measures to respond;
• to build up a strong culture of awareness and compliance with plant and food biosecurity for those with responsibilities in all sectors of agriculture and food production by harmonizing expertise across the network and by organizing trans-national and multi-sector training courses.
In addition the project tackled two cross-cutting objectives:
- to improve awareness in stakeholders and general public in biosecurity issues taking into account that information sharing is a critical issue as well as the balance between confidentiality and public access (dual use issues);
- to overcome the fragmentation of partner's research, by coordinating, monitoring and structuring the Joint Programme of Activity of the network of excellence, thus ensuring a dynamic and efficient governance of the project.

Project Results:
The considerable amount of research promoted by the European Union – which has also involved non-EU countries, such as the United States, Israel and Turkey – brings to the development of a comprehensive set of tools for project end-users. Besides them other project results include regulatory analysis and challenges identification, experimental and modeling approaches applied to plant disease epidemiology, molecular approach for diagnostics and – more generally - the outcomes of training, dissemination and networking activities aiming to increase awareness on plant biosecurity and food safety among agronomists and food producers and within the scientific, policy and inspection areas.
LIST OF TARGET CROPS AND TARGET PATHOGENS
The project established a list of pest including 570 harmful organisms likely to reduce crop biosecurity and of a list of target plants and crop products including 451 crops categorized into 11 groups (field crops, vineyards, orchards, vegetable crops, nursery and ornamental horticulture, medicinal and aromatic plants, forest production, beverage crops, straw, tree sap, seeds).
Criteria for prioritization have been identified as well. They refers to the crop economic importance (cultivated area, mean yield, mean price, value of production, volume of trade export, value of trade export,

volume of trade import, value of trade import), sociological importance (No of EU countries concerned by the crop, No of farms concerned by the crop, territorial density), consumption impact (importance in cooking traditions, importance in feeding) and environmental impact (significant presence in recreate areas, significant presence in threatened and protected areas).
A short list of important pests (63) and a short list of important target crops (21) was established using these criteria.

ADVANCES IN THE UNDERSTANDING OF THE EPIDEMIOLOGY OF DIFFERENT MODEL DISEASES

Strategies for securing plant resources must address both prevention and preparedness, led by scientific knowledge of plant disease development. The characterization of the early development of a plant disease epidemic from initial, or multiple, contaminations is a fundamental step towards the identification of a deliberate introduction. The partners worked on epidemiological knowledge of Fusarium proliferatum/Onion pathosystem and on epidemiological studies about primary inoculums of two most damaging European wheat diseases (Puccinia triticina and Zymoseptoria tritici) to assess the build-up, persistence and release of primary inoculum and the early stages of epidemics of selected pathogens to differentiate between the consequences of natural and deliberate field contamination.
The pathosystem Fusarium proliferatum (FP) - Allium cepa was selected as emerging disease with actually reported outbreaks that can offer excellent case studies of the “epidemic chain”. F. proliferatum is a soilborne fungus that infects some annual agricultural crops, including corn, rice, wheat, sorghum, onion and garlic. F. proliferatum was first isolated and identified in Israel from white onion fields in 2008 along the southern part of the Syrian African Rift. F. proliferatum may cause significant crop losses during the storage of onion bulbs, garlic heads, and corn and it can produce several mycotoxins in the tissues of infected plants, including the edible parts, including fumonisins, fusaroproliferin, fusaric acid and beauvericin. The work lead to the development of a functional model for pathogen establishment, disease and spread that identifies vulnerabilities and critical control points.
Detecting deliberate introduction of inoculum from field observations requires the assessment of the probability of obtaining these observations under the null model i.e. an epidemic without deliberate introduction.
As early stages of epidemics can be investigated most easily using a well-known disease the two most damaging European wheat diseases (Septoria leaf blotch, caused by Zymoseptoria tritici; brown rust, caused by Puccinia triticina) have been selected to support specific epidemiological studies about primary inoculum. Both diseases were chosen as experimental pathosystems to simulate the emergence of a disease (harmless to the environment) and propose a relationship between epidemiology and biosecurity, which is an issue that in its very principle excludes studying a pest in unconfined conditions.

TOOL FOR THE PRIORITIZATION OF HUMAN PATHOGENS ON PLANTS

Repeated outbreaks of human illness attributed to the contamination of fresh produce and other plant-derived foods by human pathogen on plants (HPOPs), i.e. human enteric pathogens such as Shiga toxin-producing Escherichia coli and Salmonella spp. have been analyzed in order to provide guidance on the prioritization of the risks involved in any future HPOP incident.
There are many pathogen/plant combinations that could be involved in a major contamination incident but not all are likely to have a significant impact on consumers. Whilst rigorous risk assessments can be undertaken, a relatively quick and simple approach is to consider the Political, Economic, Social, Technological, Legal and Environmental (PESTLE) factors associated with a threat scenario that allows rapid evaluation of the consequences of a contamination event. The so called PESTLE approach has been adapted and applied in this project to examples of Human Pathogens on Plant (HPOP) incidents and is

suggested as a method for prioritisation of issues/scenarios.

ANALYTICAL METHODS FOR MICROBIAL OR TOXIN CONTAMINATION IDENTIFICATION

Number of people being infected or intoxicated due to foodborne illness outbreaks reaches to millions annually. However, it is common for these outbreaks to stay unascertained or unreported by public health officials and uninvestigated by epidemiologists to trace back the source pathogen and/or food item consumed.

Fresh produce is a group of products including fruits, vegetables, herbs, and seeds and nuts, which can be consumed in form of whole, prepared (pre-cut or reduced in size), ready to eat (requiring no preparation before consumption) and/or dressed (pH controlled or not). Fresh produce can be contaminated with pathogens via environmental agents such as water, soil dust or insects during pre-harvest, and via contaminated water or cross contamination (equipment, surfaces, handlers, etc.) during the post-harvest. Since there is no inhibition step for pathogens (e.g. heat) before consumption, it is crucially important to hinder pathogen contamination rather than reducing the pathogen load on the produce. From the available data, the most common foodborne pathogens on fresh produce are pathogenic E. coli serotypes (mostly O157 – H4 and H7-, less commonly, O121, O26) and Salmonella enterica serotypes. However Listeria monocytogenes and Clostridium spp. are not common but still pose a significant threat to public health with their high mortality rates.

Although rarely causing foodborne outbreaks via fresh produce, mycotoxins are still a risk factor for foodstuff of plant origin, especially for seeds and nuts.

Methods available in the EU and associated nations for assessment of foodborne contaminants from exemplar food matrices, and the analytical methods available for microbial or toxin contamination identification, were critically reviewed. Current standards for mycotoxin analysis in food with an emphasis on minimum assay requirements for applications in a biosecurity context have been analyzed. In addition food microbiology laboratories in the EU and associated nations that could be responsive to an outbreak of foodborne illness were identified.

FORENSICALLY VALID MICROBIAL STRAIN DISCRIMINATION TECHNOLOGY FOR A FOODBORNE PATHOGEN

Non-O157 Shiga toxin-producing Escherichia coli (STEC), emerging foodborne pathogens of growing concern worldwide, have been associated with several recent multinational outbreaks of foodborne illness. To identify an outbreak as quickly as possible, to accurately link the contaminated food vehicle(s) to the outbreak, and to reduce the number of future infections are all critical components of an epidemiological/forensic investigation of an outbreak.

Currently, pulsed-field gel electrophoresis (PFGE) is the gold standard bacterial subtyping (strain discrimination) technique used in these investigations; however, it takes 2-3 days to complete the analysis. A more rapid and sensitive molecular-based bacterial strain discrimination method is needed for timely outbreak identification and contaminated food source trace back.

Multiple locus variable-number tandem-repeat (VNTR) analysis (MLVA) is a PCR-based strain discriminatory method based on the polymorphisms in multiple VNTR locations throughout a bacterial genome, and, when used as an assay, it can be completed in less than 6 hours. As a much more rapid method, MLVA is being used with increasing frequency in foodborne illness outbreak investigations to augment PFGE; however, no MLVA assay had been developed before for the emerging non-O157 STEC group. Therefore, the project developed a MLVA assay for intra- and inter-serogroup discrimination among 6 major non-O157 STEC serogroups that have been implicated frequently in recent outbreaks worldwide: O26, O111, O103, O121, O45, and O145.

DECISION TOOL TO DETERMINE WHETHER A FOODBORNE ILLNESS WAS INTRODUCED INTENTIONALLY

A foodborne illness outbreak is defined as the occurrence of two or more cases of a similar illness resulting from the ingestion of a common food. Each year, large numbers of foodborne illness outbreaks are reported worldwide. Most of these were considered to be “natural” or “accidental” events, in which the presence of the causal agents in the food vehicle was believed to be the result of improper handling. To date, there have been only two confirmed and well-documented foodborne illness outbreaks attributable to intentional contamination in the world: the salmonellosis outbreak at The Dalles, Oregon, in 1984 and the shigellosis outbreak in Dallas, Texas, in 1996.

The infrequency of known intentional events may indicate that foodborne illness outbreaks due to intentional contamination are rare, or that there is difficulty in identifying such events. Insufficient data has been the main obstacle in identifying a biological attack (bioterror or biocrime) in general and this factor may be more pronounced with foodborne illness outbreaks. Unlike most biological warfare agents, the biological agents causing foodborne illnesses, such as Salmonella and Shiga toxin-producing E. coli, are routinely carried by animals and are easily and frequently encountered in nature.

Nevertheless, the ability to differentiate between “natural” and “deliberate” foodborne illness outbreaks, especially in the early stages of an outbreak investigation, is very critical. Identifying hoaxes or other false claims is also important to reduce panic or unnecessary disruption to normal food trade. In this work, we have adapted and refined a tool that could be used by investigators to assess the likelihood that an outbreak of foodborne illness was caused naturally, accidentally or it has been developed intentionally.

ADVANCES IN THE UNDERSTANDING OF THE ENVIRONMENTAL, BIOLOGICAL AND SOCIAL IMPACTS OF AGROTERRORISM

Traditionally, crop biosecurity efforts have focused on preventing and responding to the natural or unintentional introduction, establishment and spread of pests or pathogens. This approach to biosecurity has motivated standardised approaches to Pest Risk Analysis (PRA), which have been developed to enable risk managers to identify, assess, manage and communicate risks of this kind.

Assessing and managing the risks posed by agroterrorism requires thinking differently about potential threats to agriculture and the food supply. Traditional approaches to crop biosecurity have focused on the natural or unintentional introduction of pests or pathogens. Agroterrorism, in contrast, although similarly a potential source of harmful invasive species, introduces the further dimension of a rational actor who chooses (to the extent possible) the conditions whereby risks of this kind are generated. For example, the choice of biological agent, the specific crops that are targeted, and the scale of the potential outbreak are all outcomes that are mediated by choices made by the perpetrators of an attack. In this light, biosecurity efforts must not only consider the biological characteristics of harmful invasive species, but also the motives and capabilities of individuals, non-governmental groups or state-sponsored organizations that may attempt to exploit vulnerabilities in agricultural, environmental and social systems with a view to achieving particular political, economic or personal goals.

To ensure that responses to possible threats from harmful organisms include both unintended and intentional releases the standard approaches to PRA should be revised to explicitly account for the motives and capabilities of potential attackers, i.e. individuals, non-governmental groups or state-sponsored organizations that may attempt to exploit vulnerabilities in agricultural, environmental and social systems with a view to achieving particular political, economic or personal goals.

RISK ASSESSMENT TOOL

Crop biosecurity focuses on preventing and responding to the accidental introduction, establishment and spread of pests or pathogens. Governments and industries limit these introductions through trade standards, regulated mitigation measures, public and private surveillance of new organisms, and outbreak control capacity.

There has been increasing concern about the deliberate release of biological agents against agriculture for criminal gain, to generate fear and/or undermine social stability. These threats (including the motives and capabilities of potential attackers) have been added to those included in conventional trade-driven pest risk analysis (agents, pathways and receptors) in order to develop a risk evaluation scheme. This is applied within a rule-based model to generate assessments across a range of cases, in a manner comparable to conventional pest risks, in order to enable rapid assessments of potential scenario-based threats. The tool combines expert-elicited information concerning the agent (pathogen), pathway (method of introduction) and agro-ecosystem (ecology, climate, vulnerability) in order to provide a comparative measure of risk.

The risk assessment tool enable rapid assessments of agro-terrorism scenarios and it has been demonstrated on almost 100 scenarios covering a wide range of potential motivations, biological agents, pathways and receptor systems in order to provide a comparative measure of risk. New scenarios can be added individually, and relatively quickly, as threat scenarios arise and their relative positions can be set against the background of the scenarios already established.

By re-evaluating the ratings of appropriate criteria to reflect a managed situation, the tool makes it possible to assess the effects of potential prevention and mitigation measures. The results indicate how the threat posed by different scenarios might be reduced and how responses might be improved.

Based on a preliminary characterization and contextualization of the threat resulting from plant pathogens misuse as anti-crop bioweapons in Europe, we determined that the most problematic agroterrorism scenarios for Europe are BW1 (state-sponsored threat to export trade), BW2 (state-sponsored threat to domestic production), BT1 (terrorism threat to domestic production and health), and BC1 (attack by activists or other groups against local production).

EUROPEAN PLANT DISEASE INFORMATION NETWORK

There are significant gaps in coordinated diagnostic communication across Europe: many state diagnostic labs have been privatised, and there are many advisory labs across Europe that do not have easy recourse to comprehensive specialist skills.

A web-based virtual diagnostic network (EUPDIS EU Plant Disease Information System www.niab.com/pfs) has been developed to facilitate international laboratory cooperation and to improve plant health surveillance. The network focuses on information and laboratory support systems rather than 'alert ' and 'action' mechanisms and is intended to complement the work of statutory and regulatory laboratories. The primary users of the network will be laboratories, although the structure also allows individuals such as agronomists and advisors to upload details and/or pictures of disease outbreaks. Providing these labs with a central information resource will enhance national capabilities to identify unusual occurrences or spot emerging problems, thus contributing to surveillance mechanisms and potentially accelerating responses. By joining the network, diagnostic labs will extend the "eyes and ears" that monitor crops.

The network allows information to be gathered, searched and reported, and also makes possible information flow between experts and field workers, ensuring the accessibility of summary information on disease outbreaks in Europe.

The network includes pathogen and host libraries; pages for identifying laboratories and their location, skills and facilities; and a "look-up" facility to find expert labs on specific pathogenic organisms. User labs

have the opportunity to upload diagnostic records and obtain summary information back from the system about current disease outbreaks in their own country and further afield. “Community” pages have being designed, allowing the network to provide information on diagnostic protocol development, current methods, training and accreditation courses, workshops and news of emerging pathogen problems.
Mycotoxins can cause severe intoxication of livestock and humans who consume contaminated. This includes lethal poisoning and various chronic health attributes Because some mycotoxins are harmful at very low concentrations, and since they play a possible role in crop bioterrorism and crime, highly sensitive methods are required for their detection and quantification in food matrices is needed. In such cases there is a need for high levels of assay standardization, validation, repeatability, and robustness. It is crucial to employ rapid and accurate multi-toxin screening tests for effective surveillance and analysis in food and feed. A combination of the cutting-edge technology with effective sample preparation can provide robust and practical answers for Mycotoxin detection. The chapter discusses the recent and updated methods and technologies for detection and quantification of mycotoxins

VALIDATION OF MOLECULAR APPROACHES TO DETECT NEW PATHOTYPES OF LEPTOSPHAERIA MACULANS

PLANTFOODSEC delivered an inter-laboratory validation of a molecular approach to detect new pathotypes of Leptosphaeria maculans, a fungal disease of oilseed rape with EU-wide significance. Control of the disease with fungicide is only partial, and cultivar resistance genes are often rendered ineffective by changes in the pathogen.

NIAB identified AvrIm 4-7 as target for detection. The deployment of major ‘R’ gene mediated resistance, where single, large-effect genes are used widely can lead to boom and bust cycles; an initially effective source of resistance can degrade and ultimately capsize after being defeated by new variants of a pathogen, sometimes resulting in dramatic yield losses. However, R genes have been deployed successfully in oilseed rape (Brassica napus) to control L. maculans and are key in mitigating yield losses when combined with fungicides. R genes regulate recognition of cognate avirulence (Avr) genes from the pathogen to mediate successful defence responses in the host.

Recent reports have indicated a breakdown of Rlm7 in previously resistant B. napus cultivars. Researchers demonstrated how virulent strains of L. maculans carried alleles of AvrIm 4-7 with substitutions at one (or multiple) bases leading to amino acid residue changes. The AvrIm4-7 locus demonstrates double recognition specificity, conferring avirulence to cultivars carrying RLM 4 and 7. The structure of the L. maculans promotes a high evolutionary potential and selection can result in the occurrence of new, virulent isolates; the proportion of virulent L. maculans strains increased from 0-36% in a four year period at a single field site, demonstrating the potential for the pathogen to adapt quickly and defeat genetic control strategies.

Rlm7 has been incorporated into a number of U.K. varieties and loss of field resistance is therefore of interest to plant breeders and growers alike. As a case study, NIAB sequenced PCR amplicons to monitor and detect differences in the AvrIm4-7 locus from L. maculans isolates collected from U.K. field sites. Four independent polymorphisms were identified in the L. maculans isolates tested, with a total of 9 corresponding haplotypes observed. This included substitutions at amino acid (AA) residue 120 (glycine to arginine, 358 bp), conferring virulence against cultivars carrying Rlm4. Three varieties were known to carry Rlm7 (DK Expower, Harper and Quartz) and as expected, isolates collected from these varieties were all observed to carry substitutions compared to the wild type (WT) AvrIm 4-7 allele (V23.1.3). Isolates collected from four non-Rlm7 varieties (PR46W21, Troy, Marathon and Cuillin) however comprised both WT and those carrying mutated AvrIm4-7 alleles. This demonstrated how isolates collected from a single variety can carry different complements of Avr alleles.

The use of this simple PCR sequencing strategy, which has been reproduced by FERA for inter-lab comparison, has facilitated monitoring of changes in the Avr4-7 locus in L. maculans and demonstrates how this technique can be used practically to screen for differences in an adaptive pathogen population. Although Rlm7 mediated resistance appears to have degraded in the UK leading to the appearance of leaf spots on susceptible varieties, durable, stem-base resistance present within Rlm7 varieties continues to provide good protection against summer stem-cankers.

MANAGEMENT PROGRAMMES AGAINST VARIOUS OUTBREAK SCENARIOS

The measures to be taken in order to prevent the establishment and spread of harmful crop pathogens have been established by identifying activities and responsibilities following pathogen introduction. In particular, PLANTFOODSEC identified international expertise for setting up contingency plans; listed resistant cultivars and alternative crops for a given pathogen; and developed containment and eradication protocols for selected pathogens.

In early stages of project implementation the regulations concerning the eradication and containment actions that can be taken in the event of accidental or deliberate introduction of non-indigenous harmful organisms have been reviewed and, by describing the scenarios of outbreak or interception from detection of a pest and along the management process, existing disconnection in regulation among the countries which may cause difficulties in policy regarding respond to deliberately introduced pathogens have been identified. A flowchart describing the hierarchy of responsibility and measures to be taken by the national and international authorities from the event of an organism's detection has been developed. The flowchart includes National plat protection organization, local and international police authorities. It also includes the cooperation with neighboring counties and EU institutions. The flowchart takes into account and includes the significance of the public and the media in such event. Two types of anticipated scenarios and the steps required for eradication have been covered. The first by national plant protection organization and the second (in rare cases) coordinated by the EU.

A plant disease outbreak resulting from an invasive pathogen can threaten a country's agricultural enterprise, economy and trade, and pose a threat to human food and animal feed. Therefore, following the detection of a new disease, the preferred response objective is elimination of invading pathogen(s). Invasive pathogen eradication requires a well prepared infrastructure and a coordinated process of early and rapid detection, identification of the pathogen, and the adoption and careful execution of an appropriate strategy.

ARO developed a flowchart of activities and responsibilities following pathogen introduction, which delineates the set of steps (detection, diagnosis, risk assessment, containment/eradication, management and recovery) needed in order to contain and eradicate a pathogen from the invaded site and to eliminate it. Since selection of the best approach in a given situation depends upon a realistic assessment of the effectiveness of various available approaches, and the feasibility for their use and success, a quantitative assessment of all the factors influencing the eradication process is recommended. An experimental model for testing and validating containment eradication protocols has been based on Fusarium proliferatum – Allium cepa pathosystem.

FORENSIC ASSAY FOR STRAINS DISCRIMINATION

Agricultural production is vulnerable to bioterrorist and criminal threats. To attribute such crimes, law enforcement agencies need forensically valid detection assays for plant pathogens of concern.

Microbial forensics is a scientific discipline devoted to analyzing evidence from a bioterrorist act, biocrime, or inadvertent release of a microorganism/toxin with a goal of attribution, linking a pathogen and/or a perpetrator to a specific biocrime or bioterrorst act. Attribution includes identifying the microbe(s) involved as well as those responsible. The components of microbial forensics described by Breeze et al. include 1) detection and identification of a pathogen; (2) bioinformatics, including genome sequencing and genetic databases; (3) strain repositories for pathogens or microbes of interest as well as their near-neighbors; (4) validation and standardization of forensic methods; and (5) rigorous attention to quality assurance steps. Because forensic casework is subject to vigorous challenge in a court of law, the rigor of standardization and validation of experimental, analytical and application methods goes beyond levels that are normal for typical research and management activities.

As plant pathogen forensics continues to emerge as a discipline, the need for establishing standard crime scene practices and evidence handling is needed, and procedures must be adapted and validated for plant pathogens. The pathosystem Fusarium proliferatum – Allium cepa served for the development of technologies for the forensic study of an outbreak, and specifically for assessing the source of the occurrence of a disease. To our knowledge, this work is the first to achieve the combination of (1) targeting of forensically relevant goals, (2) development and use of highly stringent and validated protocols, and (3) application and testing within a crop setting.

Reported first in 2008 in onion production fields in southern Israel, the disease worsened over several years but the source of the pathogen was unknown. Forensic testing provided new insights into the origin of the pathogen and contributed to better understanding of disease epidemiology.

Potential Impact:

Plant health is of global importance for sustainable and competitive agriculture and forestry sectors as well as for the protection of biodiversity and ecosystems . The full cost of plant pests is difficult to quantify as their vast negative impact is complex, affecting economic, ecological, environmental, social and health aspects.

From the few attempts at estimating the costs of plant pests, data indicate that in the US alone, crop losses to all plant pests total approximately US $33 billion and US$4.2 billion are lost each year in forest products, to which cost of control must be added to these figures . In developing countries, the extent of losses from "field-to-fork" caused by pests is estimated to reach 30-40% annually .

Referring to the global impact of Invasive Alien Species (IAS), Pimentel et al. calculated in 2000 that damages from IAS would total more than $1.4 trillion a year worldwide, representing nearly 5% of the world economy. In world agriculture, introduced pests have been estimated to cause $55 billion to $248 billion per year in losses .

The most direct economic impacts of pests on the agricultural and forestry sectors are the loss or reduced efficiency of production and the costs associated with their management, including the costs of inspection, monitoring, prevention, and response. Pressure is also often put on governments to extend financial assistance to the affected producers, which becomes ever more difficult in current economic crisis which is leading to an overall curbing of public costs.

In addition to these, trans-boundary plant pests also have significant negative impacts on global food security, which is nowadays of paramount concern and the most significant challenge facing mankind in the 21st century due to the 'perfect storm' of climate change, demand for energy, a growing population (estimated to exceed 9 billion by 2050) and increased pressure on natural resources . Although it is estimated that a 50% increase in food production will be needed by 2050, currently, a quarter of the world's crops are lost to pests.

Pests can affect ecosystems services, directly through removal of plants providing services, or indirectly through the effects of disease management activities, including pesticide application . They can also change biodiversity patterns and disrupt terrestrial ecosystems (including inland water bodies) and landscapes, with further impacts on economic and recreational activities.

While controlling pests has become even more difficult with the policy changes restricting conventional pesticide use approaches, such as the Directive 2009/128/EC on sustainable use of pesticides, in which EU member states are required to take measures to promote low pesticide use and Integrated Pest Management (IPM) approaches, current preventive measures (regulated mainly through the Plant Health Directive 2000/29/EC) present limitations.

Agriculture and the related upstream and downstream sectors are essential to the social, economic and political stability of Europe. Although farming employs only 5% of the European workforce, the European agriculture and agro-food industries account for 15% of the European Union annual gross domestic product, with production split almost equally between crops and livestock.

As a consequence of agroterrorism, losses could include the value of lost production, the costs of destroying diseased or potentially diseased products, and the costs of containment/eradication (monitoring, diagnostics, pesticides, vaccines, veterinary services, and destruction of potentially contaminated crops and animals) and possibly severe impacts on ecosystem function and services.

Natural outbreaks of disease demonstrate the destructive potential of an agroterrorist attack.

In 1996, the fungal disease Karnal bunt was discovered in wheat seeds in Arizona. As a consequence, more than fifty trading partners adopted phytosanitary trade restrictions against the United States. These trade restrictions resulted in USD 250 million in export losses, with the US wheat export market valued at USD 6 billion. In addition, control and clean-up costs are estimated to have reached USD 45 million.

The E. coli O104:H4 outbreak in Germany in 2011 highlighted the urgent need for rapid and reliable analytical methods. The total economic losses resulting from the incident have been estimated at between USD 0.5 and 3.5 billion. These losses were caused not only by the consequences of the pathogen being found, but also by misinformation about the source, the delay in determining the source pathogen and its hosts and in reporting cases, and subsequent trade bans that were not always appropriate.

Export markets could be lost, as importing countries are likely to lead to impose restrictions on EU products to reduce the possibility of disease spread.

Multiplier effects would ripple through the economy due to decreased sales by agriculture dependent businesses (farm input suppliers, food manufacturing, transportation, retail grocery, and food services and tourism). Experience has shown that products similar to those affected may be equally influenced by a significant event - though increased due diligence or — positive release‖ testing and examination may not be cost effective - can cause severe financial losses in unaffected products. Consumer confidence in government(s) might also be deleteriously affected depending on the scale of attention paid to the threat of agroterrorism as well as on the eradication effort which is potentially disruptive, requiring additional measures to control disease spread that will impact far beyond the agricultural community (e.g. disinfection of delivery and service vehicles in control zones, restrictions on animal movements, including recreational or sports animals, etc.).

The psychological impact on the community and emergency responders cannot be calculated but remains significant. Beyond the immediate economic and political impact, agroterrorism attacks could also elicit fear and anxiety among the public.

The number of lethal and contagious biological agents is far greater for plants and animals than for humans. Most of these pathogens are environmentally resilient, endemic in foreign countries, and harmless to humans, making them easier for terrorists to acquire, handle and deploy.

Plant and food biosecurity is a relatively new field of research in Europe. The majority of work has been done in the United States, where many agencies and entities within the US government, State and local government, the private sector and universities are actively engaged in protecting U.S. agricultural resources from intentional or unintentional introduction of pathogens. Countries such as Australia and New Zealand also have invested in biosecurity research, notably on how to successfully exclude, eradicate and control unwanted agents that threaten the economy, environment and human health. Several programmes, in USA as well as in Australia and New Zealand, have identified those agents posing the greatest threat, leading to a number of lists of plant and animal pathogens, compiled independently by several agencies. In Europe, Pest Risk Analysis (PRA) is well developed but has not yet focused on the risks presented by deliberate pathogen introduction.

Whatever the cause of a plant disease outbreak, a comprehensive biosecurity system is essential to protect agriculture, food and citizens. Current EU capabilities to detect and respond to agro-terrorism and bio-criminal acts are very modest. Capacities are spread among many organisations, normally regional or national bodies, and there is a lack of coordination. As biosecurity risks transcend national and regional boundaries, it is essential to monitor, assess and manage them in a coordinated way across the EU.

As a first step, PLANTFOODSEC established a virtual Centre of Competence on Plant and Food Biosecurity, which is intended as a “backbone” for the EU scientific community in the field of plant and food biosecurity. It comprises a network of research centres, universities and other stakeholders, whose aim is to enhance preparedness, response and recovery capabilities in the event of intentional and unintentional biosecurity threats to EU agriculture, farming and the agro-food industry. The PLANTFOODSEC consortium is working to make the virtual European Centre of Competence on Plant and Food Biosecurity concrete.

PLANTFOODSEC enhanced preparedness and response capabilities to prevent, to respond and to recover from a biological incident or deliberate criminal activity threatening the European agrifood system. Throughout the development of a project “toolbox” PLANTFOODSEC addressed end-users needs.

MAIN DISSEMINATION ACTIVITIES

PLANTFOODSEC has been approved under the FP7 Security call SEC-2010.7.0-1 addressing the networking of researchers for a high level multi-organisational and cross-border collaboration.

The topic expected impact was to establish a (virtual) network of excellence to increase the quality and impact of training and research in Europe in the specific security field (plant & food biosecurity). The aim of the PLANTFOODSEC Network of Excellence was to build a strong interlinked Network based on the expertise of the participants in biosecurity, including diagnostics, epidemiology, risk assessment, microbial forensics, control, containment and regulation of human and plant pests and pathogens in the crop production chain. However, the aim was not only to train the Network participants to a high level in these areas but extend the training and knowledge transfer activities to raise the awareness and compliance with plant and food biosecurity for those with responsibilities and interest in all sectors of crop agriculture, including extension specialists, students, crop consultants, regulators and farm advisors. It was perceived that the EU capacity to respond to attacks on the crop-food chain was becoming limited by shortage of skills in pivotal disciplines, including taxonomy, plant pathology and various other disciplines so training plans were developed to raise the skill level of new entrants to the profession.

To this end, a lot of effort has been devoted to training, dissemination and networking activities, although by taking into account the balance between confidentiality and public access in security research (dual use issues).

DEVELOPMENT OF DISSEMINATION MATERIA

DEVELOPMENT OF DISSEMINATION MATERIAL

PlantFoodSec tools that fell among the traditional dissemination comprised the preparation of various dissemination materials. In the five years project duration, these included the development of an internet website, three flyers, ten editions of the digital six-monthly newsletter, seven press releases and the preparation of publications in the form of PhD thesis and scientific papers, as well as articles, posters and technical communications.

To start the very first PlantFoodSec dissemination activity with a tangible communication gesture, a press conference was held in Torino, Italy, on February 24, 2011, right at the project kick-off meeting. It was attended by EC representatives and the local and national press. Focused actions with a selected number of European media took place regularly in view of major events during the project progression. All coming press releases, which were issued during the project implementation, lead to a considerable number of articles in national and international media and websites. The press releases served mainly to attract media attention to significant events and publications. A full press review is available on the project website (www.plantfoodsec.eu).

To date 21 peer-reviewed publications quoting the project have been published in scientific press in addition to 41 papers/abstracts in conference proceedings and to 3 chapters in edited books.

Moreover, a book dedicated to the project will be published by Springer in late 2016. The title is “Practical Tools for Plant and Food Biosecurity – Results from a European Network of Excellence” and it will be part of the book series “Plant Pathology in the 21st Century” http://www.springer.com/series/8169?detailsPage=titles

PLANTFOODSEC did not included in its Annex I the development of social media account, as the project has been developed in 2010. However, two social media accounts managed by the project partner Frédéric Suffert (INRA) include project news and, more broadly, news and research related to plant biosecurity and agroterrorism field, the Scoop.it account Cropbiosecurity and Agroterrorism Watch and the Twitter account @cropsafexpert https://twitter.com/cropsafexpert

PARTICIPATING IN EXTERNAL DISSEMINATION EVENTS

Project partners participated to several external dissemination events on biosecurity and research bringing the project experience. In particular, the project has been presented in the following events:

• the workshop organized in Rome on 16/03/2011 by the farmer organisation Confagricoltura

“Agroterrorismo: un rischio per la biosicurezza in campo agro-alimentare?”;

• the SECURENV project final end-user workshop “Future Security Threats and the Environment” organized in Brussels on 4/4/2011;

• the Security Research Conference 2011 organized in Poland on September 2011. PLANTFOODSEC project was present in the exhibitors area with a stand;

• the initiative “Tutti gli studenti e i docenti da settembre all’Università” attended by more than 1200 students of Grugliasco primary schools (September 2011, Grugliasco-Italy);

• the workshop “Alimenti di origine vegetale – biosicurezza e rischio microbiologico” held in Torino on 9/3/2012;

• the editions from 2011 to 2015 of the European Researchers’ Night in Torino, Italy;

• the editions from 2011 to 2016 of National plant pathology congress “Incontri Fitoiatrici” in torino, Italy

• the HOMSEC, IV Salòn International de Tecnologìas de Seguridad y Defenza, in 12-15 March 2013, Madrid Ifema, Spain;

• the 10th International Congress of Plant Pathology 24-30 August 2013 in Beijing, China;

• the "EUROPEAN BIOTECHNOLOGY CONGRESS", 16-18 May 2013, Slovakia, Bratislava;

• "Seminar on Food Safety & academic visit" in the scope of PLANTFOODSEC Exchange programs, 15-18 August 2013, Oklahoma State University;
• “Food Defense Collaborative Exchange" - NCFPD in the scope of PLANTFOODSEC Exchange programs, 19-24 August 2013, University of Minnesota;
• International Association for Food Protection Annual Meeting , 28-31 July 2013, USA, North Carolina;
• NCT Food Supply Chain Security: a European Forum , 23-24 October 2013, Brussels;
• 36th Mycotoxin Workshop held in Gottingen, Germany (16-18 June 2014) by UNIBONN;
• Improving Food Safety and Labelling in the EU: Smarter Rules for a Sustainable Agri-Food (Brussels, 9 September 2014);
• European Commission CBRN and explosives advisory group biological sub-group (Brussels, 10-11 June 2014);
• XXI Congress of Italian Phytopathological Society (Società Italiana di Patologia Vegetale - SIPAV2015) held in Torino on September 21-22-23, 2015;
• Invasive Alien Species – Czech contribution to challenges for EU research and policies (Brussels, 3 June 2015);
• Workshop “Parassiti e specie aliene invasive per le colture: necessità di una risposta integrata da parte della comunità scientifica” (Roma, 3 June 2015);
• Workshop “L’Italia e il primo anno di Horizon2020 - Societal Challenge 2: Food Security, Sustainable Agriculture and Forestry, Marine, Maritime and Inland Water Research and the bioeconomy”;
• The 2016 annual congress of the Israeli Phytopathological Society;
• 3rd Meeting of the Community of Users on Disaster Risk and Crisis Management (Brussels, February 29, 2016) by UNITO.

PROJECT WORKSHOPS

The launch workshop of PLANTFOODSEC meeting was held on October 10th, 2011 at REC (Szentendre, Hungary) with the participation of project partners speakers and of external speakers of the EU funded project AniBioThreat and SECURENV.

The mid-term workshop took place in China during the International Congress of Plant Pathology in 2013 http://www.icppbj2013.org with the theme Bio-security, Food Safety and Plant Pathology: The Role of Plant Pathology in a Globalized Economy (August 25-30 2013 Beijing, China).

A roundtable on Plant and Food Biosecurity was held in Brussels on 7 February 2013, Belgium.

A workshop dedicated to end-users (“Virtual diagnostic network and risk assessment tool for Plant Biosecurity”) has been held at REC (Szentendre, Hungary), on April 22nd, 2015.

The closing workshop “Tools for Plant and Food Biosecurity” has been held in Brussels on January 19th, 2016.

TRAINING COURSES

During the project a total of 31 training courses were held on preventing, preparing for, containing, and responding to bioterrorism and/or naturally occurring disease outbreaks: 25 courses targeting students and 6 courses targeting public administration, policy makers and scientists. 16 courses have been held in Italy, 7 in UK, 2 in USA and 1 course each in France, Israel, Turkey and The Netherlands.

A total of 28 courses on diagnostic at laboratory and field level has been carried out targeting students at undergraduate and PhD level (9), trial operators and breeders (4), researchers (9), policy and inspectors (3), seed companies and agronomists (3). Nine courses have been held in UK, six in Italy, six in Germany, 2 in Turkey and 1 in USA.

Concerning plant pathogens forensics a total of 47 courses have been held for students (25 courses),

scientists and professionals (18), general public (2) and defence community (2). Most part (30) of them have been held by OSU in USA, 3 in Turkey, and 1 each in Sweden, Canada, India and Mexico.
Finally, two seminars were given in UK to plant health inspectors on legislation and contained use licensing.
PLANTFOODSEC SUMMER SCHOOLS
The first summer school was held in Cambridge (NIAB) in September 2011 dealing with principles of Plant Disease Identification. The second summer school was held in Torino (UNITO) in July 2012 dealing with Plant and Food Biosecurity.
The third summer school on Diagnosis of plant pathogens (light microscopy, remote sensing up to hyperspectral imaging techniques as well as conventional techniques for identification up to TEM and REM techniques) should have been held in Bonn (UNIBONN) in Summer 2013 but has been cancelled and replaced by two courses held in York at FERA: one course on Field and lab diagnosis of plant pathogens using LAMP and microarray methods in February 2015 and one course dedicated to Diagnosis of plant pathogens in field - Mycotoxins –chemical/immulogical methods on July 2015.
Another summer school was expected to be organised by INRA and ANSES in France on July 2014. However it has not been completed due to the lack of applications.
Last PLANTFOODSEC summer school has been organised by METU on Subtyping methods for Human Pathogens on Plants on September 2015.

List of Websites:
www.plantfoodsec.eu

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