European Cooperation in the field of Scientific and Technical Research - COST -

Secretariat

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COST 258/08

MEMORANDUM OF UNDERSTANDING

Subject :	Memorandum of Understanding for the implementation of a European Concerted Research Action designated as Cost Action FA0807: Integrated management of phytoplasma epidemics in different crop systems

Delegations will find attached the Memorandum of Understanding for COST Action FA0807 as approved by the COST Committee of Senior Officials (CSO) at its 172nd meeting on 24-25 November 2008.

MEMORANDUM OF UNDERSTANDING

For the implementation of a European Concerted Research Action designated as

COST Action FA0807

INTEGRATED MANAGEMENT OF PHYTOPLASMA EPIDEMICS IN DIFFERENT CROP SYSTEMS

The Parties to this Memorandum of Understanding, declaring their common intention to participate in the concerted Action referred to above and described in the technical Annex to the Memorandum, have reached the following understanding:

1. The Action will be carried out in accordance with the provisions of document COST 270/07 "Rules and Procedures for Implementing COST Actions", or in any new document amending or replacing it, the contents of which the Parties are fully aware of.

2. The main objective of the Action is to promote information exchange in order to design integrated phytoplasma management strategies for the sustainable production of high-quality plant products and to reduce pesticide use resulting in less residues in fresh market products fruit, vegetables and grapevine.

3. The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 108 million in 2008 prices.

4. The Memorandum of Understanding will take effect on being accepted by at least five Parties.

5. The Memorandum of Understanding will remain in force for a period of 4 years, calculated from the date of the first meeting of the Management Committee, unless the duration of the Action is modified according to the provisions of Chapter V of the document referred to in Point 1 above.

A. ABSTRACT AND KEYWORDS

Phytoplasmas are insect-transmitted plant pathogenic prokaryotes causing serious diseases in important crops such as grapevine, vegetables, corn, sugar beet, oil-seed crops and fruit trees throughout Europe. Recent advances in phytoplasma genomics have generated an impetus for research into control and management of these diseases. This Action will integrate European phytoplasma research, enabling research funds to be used more efficiently by reducing duplication of efforts. New approaches for disease management based on understanding the phytoplasma-plant interaction at a molecular level will be achieved. This will result in improved diagnostic methods; reduction of disease spread; improvement of insect-vector monitoring and a reduction in the pesticides used for control. Knowledge-transfer will be enhanced through meetings, workshops for agro industry, website, newsletters and scientific publications. Early-stage researchers and scientists from COST countries will be encouraged to participate in lab exchanges, and public understanding of phytoplasma diseases will be increased. The goal of this Action is to promote information exchange in order to design integrated phytoplasma management strategies for the sustainable production of high-quality plant products and to reduce pesticide use, resulting in less residues in fresh market products: fruits, vegetables and grapevine.

Keywords: Crop epidemics, phytoplasma, diagnostics, epidemiology, vectors, sustainable disease management.

B. BACKGROUND

B.1 General background

Phytoplasmas are non-culturable, phloem-limited, insect-transmitted plant pathogenic prokaryotes, and their genomes are among the smallest known (530 - 1,200 kbp). They cause devastating diseases in many economically important crops in Europe: grapevines (e.g. grapevine yellows, 'bois noir', 'flavescence dorée'), potato (potato stolbur), tomato (tomato big bud), sugar beet, corn, oil-seed crops, stone and pome fruits (e.g. European stone fruit yellows, apple proliferation, pear decline) are the major economic crops that suffer considerable losses due to these pathogens, both in pre-harvest yield, and in post-harvest quality. As an example, grapevine 'flavescence dorée' (which is a quarantine pathogen) causes epidemics in parts of Italy, France, Spain, Switzerland, and Serbia with losses ranging from 10 to 80% of the grape production. In addition, production losses reaching 50% are sometimes found associated with a second phytoplasma, the 'bois noir' phytoplasma. Apple is also an important European crop, and the total apple production in Europe in 2001 was 7.6 million tons where the main producing countries were Italy (2.2 million tons), France (1.9 million tons) and Germany (922,000 tons). The average sales revenue for apples in Germany in 2001 was about 270 Euro per ton, which resulted in a total revenue of about 250 million Euro. Observations in German, French and Italian apple orchards showed damage due to apple proliferation ranging from 10-70% of the total harvest, and it has been calculated that the disease caused a loss of about 25 million Euro in Germany and of about 100 million Euro in Italy in 2001 alone. Among stone fruit trees, apricot is the most affected species by phytoplasmas. Apricot production in Europe in 2002 was 578,000 tons with the main production markets being Italy (207,000 tons) and France (170,000 tons). In Eastern Europe, the southern part of the Czech Republic is an important production area, and whilst the average harvest between 1998 and 2002 was about 3,330 tons, the harvest for 2003 increased to 5,031 tons reflecting the rising importance of apricot production in this area. The mortality of apricot trees due to phytoplasmas ranges from 7 to 30% with an average production loss of 10%, and phytoplasma disease is the biggest obstacle restricting apricot replanting and expansion of the orchards, and causes significant economic damage for fruit farmers and nurseries. Phytoplasmas are likely to be of greater importance in the

future because of climate change, with the range of their insect vectors expanding to northern European regions, combined with the ability of these vectors to survive in larger numbers during mild winters. In addition, the phytoplasmas are more readily able to replicate in infected plants in warmer conditions, such that the symptoms they produce are likely to be more severe and devastating.

Current control options

Prevention of phytoplasma infection is the primary control strategy, which comprises quarantine measures, eradication of diseased plants, and hygiene in plant propagation as well as vector control strategies. A vital pre-requisite in these control measures is the availability of efficient and sensitive pathogen detection methods, and there has been significant progress in this area because of the rapid advances in phytoplasma genomics, and it is timely to build on these advances to improve methods further, and understand more about phytoplasma biology. There are currently no effective chemicals or strategies for eradicating phytoplasmas from plants in the field, but the greater understanding of phytoplasma biology that results from this Action might change this. Whilst the use of chemicals to control phytoplasmas themselves is currently not possible, chemicals can be used to control vectors and reduce disease spread. However, there is increasing public pressure to reduce chemical inputs, such that an understanding of vector ecology is vital towards making more efficient use of chemical control and reducing chemical inputs.

Why a COST Action?

Phytoplasma diseases have the potential to rapidly spread across European regions through their insect vectors (which can readily cross borders) or through transport of infected plant material. The phytoplasma research community comprises well-established research groups distributed throughout Europe (including new EU Member States) and national funding programs are enabling these research teams to undertake fundamental research on phytoplasmas. However, there is a need for research coordination to consolidate these limited national funding resources, reduce duplication of efforts and maximize the efficiency of developments for the

enhanced control of phytoplasma diseases. Essential trans-European resources such as phytoplasma *in planta* and *in vitro* culture reference collections will be managed through the Network, along with essential training and transfer-of-knowledge from scientists in countries that have experience with phytoplasma diseases to countries not yet exposed or with limited resources. This Action strongly supports transfer-of-knowledge from experienced scientists to early-stage researchers, breeders, growers and industrial partner organizations and scientists from less developed countries. In the 1980s and 1990s there were a number of EU Frameworks and INCO-DEV projects on phytoplasma diseases mainly directed toward epidemiology and diagnostic studies. The recent advances in phytoplasma genomics, combined with the increased threat from phytoplasma diseases, has provided the focus and impetus to bring together the European phytoplasma research groups into a coordinated Action: a non-competitive, international platform such as COST is the ideal vehicle to support and strengthen this networking. Lack of international coordination has the potential to result in a missed opportunity, and the spread of damaging phytoplasma diseases to hitherto unaffected countries or crops, resulting in significant crop losses. Conversely, international coordination of research through a COST Action will provide scientists and ultimately practitioners (farmers and agro-industries) with the tools to rapidly detect and monitor the spread of these pathogenic organisms, and implement control/eradication strategies.

B.2 Current state of knowledge

During the past 5 years, the genomes of 6 phytoplasmas have been completely sequenced (four were already published) and a number of others are partially available. Whilst the majority of these projects have been led by research groups from outside Europe, European research groups have been involved in some of them. Studies are also in progress towards identification of plant genes involved in phytoplasma pathogenicity and in host-pathogen interaction. These studies have provided insights into fundamental aspects of phytoplasma biology, and since the genomes of phytoplasmas are among the smallest known in nature, and are recognized as representing an example of the minimal gene set required for life, the results are providing valuable information both from a plant pathology and from a fundamental biology perspective.

From an innovative perspective, these genomics data are providing valuable resources and information for improving diagnostics and classification of phytoplasmas, and as outlined above, these informations are essential prerequisites for improving disease management.

The key innovations will be: new diagnostic methods which give improved taxonomic identity and quantification of the pathogen; standardization of culture collections to facilitate biodiversity studies of phytoplasmas and to identify model systems for coordinated studies; application of systems biology to the study of phytoplasma/plant interaction; a single user-friendly database of European phytoplasma/host/vector details including bioinformatics information useful to identify potential vector genera/species and weed hosts; establishment of a vector/disease monitoring network. The novel approaches will be useful to identify new phytoplasma host plants, unknown vectors of important diseases and to improve diagnostics, establishing quicker, more economic and robust methods to use especially for producing certified plant material. Information will also be acquired about metabolic pathways that can lead to identification of genes involved in pathogenicity mechanisms, useful for increasing plant resistance to phytoplasma diseases. This will help in disease management and production of phytoplasma-free seedlings as well as in providing scientific knowledge that can be used to inform policy makers in the implementation of improved European-wide quarantine rules and regulations.

B.3 Reasons for the Action

The recent advances in phytoplasma research combined with the increased threat from phytoplasma diseases due to climate change has resulted in a drive to bring together the European phytoplasma research groups into a coordinated Action. This will result in improved and harmonized diagnostic, detection and monitoring systems for phytoplasma diseases and for their vectors, which will aid plant health inspectors and quarantine services, and result in improved and less environmentally damaging approaches for managing phytoplasma diseases, to help maintain safe and secure European agriculture and food production. Achievement of basic information about host-phytoplasma interaction will also result in designing strategies to reduce impact of phytoplasma diseases in European agro-industry systems.

The Action is mainly aimed at European economic/societal needs enhancement of the competitiveness of European agro-industry, production of healthier and high quality fresh food products. The scientific/technological advances will be achieved as well through implementation of novel technologies in phytoplasma disease management.

B.4 Complementarity with other research programmes

There is complementarity with the 6th Framework Project EUPHRESCO (Phytosanitary ERA-NET www.EUPHRESCO.org), which started on 1st May 2006 and will continue until 2010. EUPHRESCO is a partnership of 24 leading organizations involved with funding phytosanitary research in 17 European countries.

There is complementarity with the project EUREKA Project E1 3233, EUROAGRI + PSYLLID VECTORS “Development of efficient control strategies against psyllid species vectoring European fruit tree phytoplasmas" which runs from July 2005 until October 2008 and includes 8 Countries and 13 different research groups.

There is complementarity with SEE-ERANET PHYTOPLASMA-EPIDEMIO network (Global epidemiology of phytoplasma diseases of economic importance in Southeast Europe) which runs from Nov 2007 to July 2008, and includes 8 Countries and 13 different research groups.

There is complementarity with the INTERREG IIIA OMS 3a.5/PAM 1.2.4. "Etablierung eines Warndienstes zur Bekämpfung der Besenwuchs-Krankheit des Apfels (Apfeltriebsucht)", "Mise en place d’un systéme d’alerte pour le controle de la maladie de la proliferation du pommier" Jan 2005-July 2007. This project integrated the website on apple proliferation and the psyllid key.

There is complementarity with the project: “Improvement of identification methods based on molecular characterization of stolbur phytoplasma strains causing serious diseases in horticultural crops” under the XVII executive programme of scientific and technological co-operation between the Republic of Hungary and the Republic of Italy for the years 2008-2010.

C. OBJECTIVES AND BENEFITS

C.1 Main/primary objectives

The main objective of this Action is the enhancement and exchange of scientific knowledge and technologies related to phytoplasma diseases, through the establishment of a multidisciplinary scientific European Network, aimed at developing strategies to detect and prevent biological invasion, and the spread of phytoplasma diseases of plants.

C.2 Secondary objectives

The Action will strengthen and consolidate existing European networks through the following secondary objectives:

- develop and optimize diagnostic strategies/protocols based on innovative molecular tools for phytoplasmas in the European Union;
- establish core collections of phytoplasma strains for use in development of rapid diagnosis of disease and for reference;
- establish a network for vector population monitoring;
- design integrated and innovative management strategies for sustainable disease control;
- promote an international response to disease threats by networking countries on the front-line of disease outbreaks with vulnerable, but disease-free countries;
- promote a basic understanding of diseases by establishing a systems biology platform;
- support national plant protection organizations and European organizations (e.g. EPPO) by providing data and extension materials on phytoplasma diseases;
- transfer innovations rapidly to end-user groups (i.e., SMEs, plant protection organizations, plant inspection services, grower groups);
- train early-stage researchers and scientists from less developed regions of the COST countries with the most friendly and innovative technological knowledge;
- provide training of plant protection officers in countries threatened by biological invasion by diseases (including diagnostic, monitoring and quarantine procedures);
- increase public awareness of phytoplasma diseases.

Emphasis will be placed on filling gaps in national awareness of phytoplasma disease threats, and on procedures to rapidly detect and combat phytoplasma invasions in European agro-ecosystems.

C.3 How will the objectives be achieved?

The objectives will be achieved through close cooperation of the laboratories involved. Working Groups will be established that hold regular meetings enabling rapid presentation and discussion of recent scientific data. The proceedings of these meetings will be published on a dedicated website that will be established through this Action, and a Newsletter with graphic fact-sheets describing diseases and control/prevention options will be produced to alert other interested parties (e.g. EPPO, grower groups, plant inspection services, agro-industries etc.). The website will also provide bioinformatics data in a standard format to facilitate dissemination of collaborative progress. Public awareness of phytoplasma diseases will be enhanced through publication in non-scientific journals, and in an open access website domain. Exchange programmes for early-stage researchers and scientists with short term scientific missions (STSMs), exchange of materials, and training workshops in diagnostics and in other key methodologies will be managed through the Action.

The progress of both primary and secondary objectives will be monitored mainly through progress reports and the evaluation of milestones listed in part E.

C.4 Benefits of the Action

To the scientific community: the main benefit of this Action will be to increase the competitiveness of European phytoplasma research in the world arena. The results of this will be accelerated research progress, increased publications and the development and transfer to end users of strategies to control these diseases. The multi-disciplinary nature of the Action, will enable researchers from a range of disciplines (e.g. pathologists, geneticists, diagnosticians, molecular biologists, entomologists) who share a common interest (phytoplasma), but do not regularly interact at scientific conferences, to meet and exchange innovative ideas, and will in turn lead to the establishment of new networks and further EU research projects (e.g. FP7).

To society and the environment: understanding mechanisms of phytoplasma diseases will result in improved methods for disease control, including a more targeted and effective use of insecticides, such that there overall usage and the environmental damage they cause, is reduced. The reduction in crop losses will improve agricultural productivity which in turn will help drive down the costs and increasing the quality to the consumer for foodstuffs.

For employment: a concerted European Action on phytoplasma diseases and the increased competitiveness of the research that results from this will not only improve the skill bases of phytoplasma researchers across Europe, but will also ensure that future opportunities are available for early-stage researchers to pursue careers in this area.

For European research: this Action will include a large number of former Eastern European countries where agriculture and the rural economy employs significant numbers of workers, and where phytoplasma diseases have a major impact on productivity. The integration of researchers from these countries into the framework will ensure that they can obtain the best and most relevant and innovative training in new technologies. In turn, the old COST countries will gain a greater understanding of the distribution of phytoplasma diseases and of their vectors in the new COST countries, and will be better able to monitor the spread of these diseases and protect the agricultural industries against disease spread across Europe, increasing in the same time the quality of food stuff.

For developing nations and the global economy: phytoplasma diseases are a significant problem in developing countries such as Africa, Asia and South and Central America. European researchers have been actively involved for many years in phytoplasma research in these countries through EU and National Government funded programmes, and there are current projects that address the Millennium Development Goals. The enhanced competitiveness of European research into phytoplasmas with innovative methodology that results from this Action will maintain and improve the position of European scientists to contribute to research programmes and collaborations with scientists from developing countries, targeted at improving agricultural productivity and addressing these Millennium Development Goals.

C.5 Target groups/end users

The improvements to diagnostic, detection and vector monitoring systems will aid plant health inspectors and quarantine services to reduce the risk of spread of phytoplasma diseases into and within Europe. Appropriate and coordinated use of control methods will improve crop management and provide financial benefit to the farming and agricultural industries both in the COST countries and in developing countries. An improved understanding of fundamental phytoplasma biology will enhance the competitiveness of European research scientists and also allow the availability of germplasm material that can have reduced impact from phytoplasma diseases.

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

To achieve the different objectives, the scientific programme has been discussed amongst all the scientists who have expressed an interest in taking part in this Action to date. The effective control and management of phytoplasma diseases requires integration of diverse scientific data that needs to be acquired by scientists with a range of different skills and disciplines. Diagnosticians and taxonomists are required to determine the nature of the diseases already present in Europe, and to identify any new phytoplasma isolates and disease threats quickly and precisely. Such studies will be linked to the work of entomologists who can provide effective identification of vector species and a coordinated and continuous monitoring of vector populations. Plant scientists, field pathologists and advisors are constantly improving disease control methods, using practical preventive measures such as improved cropping practices and/or producing effective barriers against the vectors. Plant breeders, geneticists, molecular biologists and bioinformaticians are developing an understanding of the molecular basis of phytoplasma pathogenicity, and of disease resistance mechanisms in plants, which will ultimately feed into novel disease control strategies based on plant breeding and biotechnology. Four Working Groups (WGs) will be established focusing on diagnostics, vector monitoring, control strategies and host/pathogen interactions, and

the data from the four WGs will be fed into a dedicated website and discussed through a programme of meetings and scientific exchanges. The website will be the focal point of the Action, integrating and disseminating information between the partners that can be used for the effective management of phytoplasma diseases in different crop species, whilst at the same time providing a public domain to increase public awareness and potentially identify new hosts and diseases.

D.2 Scientific work plan – methods and means

Working Group 1: Early detection and diagnostics

Early and sensitive detection and diagnosis of phytoplasmas is of paramount importance for effective prevention strategies, particularly because phytoplasmas may have a very long latency period. The main objectives of this WG are to compare diagnostic procedures already available for most phytoplasma pathogens and/or develop novel methods and integrate these into sensitive and simple early detection protocols, suitable for monitoring propagation material and for screening in plant-inspection services. To accomplish the goals in this task marker genes that show sufficient polymorphism will be selected as DNA bar-coding regions, and a database of available collections of phytoplasma strains and/or DNA will be established.

Task 1 will establish a database of available collections of phytoplasma strains or DNA collections in the COST countries, and make these available to researchers. Specimens in these collections will be chosen as standards for diagnostic purposes. As the maintenance of phytoplasmas is difficult and time-consuming, alternative methods such as amplification of stored DNA will be explored.

Task 2 will identify suitable marker genes for differentiation at species and sub-species level (bar-coding). These genes will form the basis of new diagnostic protocols. Links will eventually be established to projects within the newly launched program KBBE-2008-1-4-01: Development of new diagnostic methods in support of Plant Health.

Task 3 will apply the above mentioned collections and bar-coding regions to study pathogen diversity throughout the COST countries and neighbouring countries.

Task 4 will develop new detection protocols for selected phytoplasmas and optimize and validate these and existing protocols. The protocols will be based on molecular techniques such as real-time PCR, diagnostic microarrays etc. The work will include all steps from sampling, DNA extraction to interpretation of results.

Task 5 will make standardized protocols based on results from the above tasks in the WG that can be used by practitioners such as plant inspection services and producers of propagation material. These standardized protocols will be disseminated after final evaluation for reliability and robustness.

Working Group 2: Epidemiology and vector ecology

Epidemiology will study the dispersal of phytoplasma diseases. Phytoplasmas are transmitted in a persistent manner by insects belonging to the families Cicadellidae, Cixidae, Psyllidae, Delphacidae, and Derbidae. The vector acquires the phytoplasma by feeding on an infected plant and then transmits the pathogen to a healthy one only after completion of the latent period, during which phytoplasmas multiply in the midgut, haemocytes and salivary glands of the vector. Factors influencing the length of these periods as well as the efficiency of transmission will be studied. Once a vector becomes infectious, infectivity is retained for life, although some discontinuities in vectoring abilities have been reported for several phytoplasma-vector associations that will also be investigated. Some factors influencing transmission, among which are life stage, gender, presence of associated symbionts, flight behaviour, weed control measures, temperature, phytoplasma strain, source and recipient plant species will be studied for relevant phytoplasmas. Pathogenicity effects on different organs or even reduction of longevity and fecundity will be studied for selected phytoplasma-infected vectors. Although phytoplasmas have been detected in various organs and tissues of the vectors, the existence of two barriers has been suggested: the midgut and the salivary glands. There are reports of phytoplasma multiplication in the midgut of nonvector insects, clearly indicating that there are cases where phytoplasmas colonize the insect but are not transmitted. Moreover in some cases, even the host plant may influence the outcome of transmission. In fact certain plant species may be infected with phytoplasmas by feeding insects, but are unsuitable for further acquisition, at least with some vector species.

Micropropagation together with other agricultural practices such as grafting, cutting, stool bed and other systems to propagate plant germplasm avoiding sexual reproduction are other known ways for transmitting phytoplasma diseases, and recently the possibility of transmission through seed has also been under investigation. In apple, transmission by natural root bridges may be of underestimated importance as well.

The objectives of this WG are to establish a vector monitoring system throughout Europe to identify phytoplasma vector species, monitor their spread throughout the COST countries, and to co-ordinate research into these and other means in which phytoplasmas are spread.

Task 1 will establish tools to identify vector species e.g. psyllid species. For this an internet-based electronic determination key for putative psyllid vectors will be established (based on the key developed in the INTERREG IIIA project OMS 3.a). In addition, molecular markers for the identification of vector (psyllid) species (linked to a EUREKA project) will be developed and validated. Both tools will be combined and accompanied by a collection of the most important reference specimens in order to provide an exhaustive tool for specific vector determination.

Task 2 will be the establishment of a database to monitor the presence of phytoplasma diseases and of their putative vectors in defined regions throughout Europe.

Task 3 will provide data about the infectivity of a vector species using molecular quantification methods. These data will help to differentiate between vector and non-vector species and will lead to the establishment of a risk assessment system based on the number of potentially infectious vector individuals in a defined area.

Task 4 will collect data on the differences in vector populations throughout European regions by using both biological and molecular (SSCP) methods. There is a link to a SEE-ERA.net pilot project dealing with the molecular differentiation of vector populations. The data will provide correlations between vector populations and efficiencies in disease spread in different European regions. Studies will be performed on life history of vectors with particular respect to biotic and abiotic factors affecting vector abundance and on determination of the potential range of vector species due to diverse climatic conditions.

Task 5 will evaluate the influence of different means of disease spread other than vector transmission e.g. seed transmission or transmission by root bridges.

All data collected in tasks 1-5 will be used to give recommendations for the tasks of WG3 in order to develop efficient control strategies for specific pathogen systems.

Working Group 3: Crop systems and control

Control of epidemic outbreak can be carried out theoretically either by controlling the vector or by eliminating the pathogen from the infected plants by antibiotics (mainly tetracycline, due to the lack of a cell wall in phytoplasmas) or by other chemicals. However, these protection measures have proved to be quite ineffective under field conditions, firstly because it is impossible to eliminate all vectors from the environment, and secondly because the use of antibiotics is very expensive, not allowed in several countries, and not always effective over the long-term since they do not eradicate the phytoplasmas, such that repeated treatments are necessary. Therefore the only effective way to control phytoplasma infection has been to prevent the outbreaks by ensuring that clean planting material is used, or by endeavouring to find and/or breed varieties of crop plants that are resistant or tolerant to the phytoplasma/insect vector. In order to advance this field of research basic knowledge about the epidemiology, the pathogenicity mechanisms of the phytoplasmas, the effects of environmental factors on disease and symptom development, and the nature of resistance/tolerance in host plants is required. The recent sequencing of phytoplasma genomes has provided evidence

that small peptides secreted by phytoplasmas are able to enter plant cells and move between cells, and that some of these secreted peptides are likely to be key pathogenicity factors, and may therefore be potential targets for plant defence mechanisms. Identification of alternative control strategies against these diseases, such as the possibility to use biocontrol organisms or phytoplasma mild strains could also provide innovative and promising tools for limiting phytoplasma spread in an environmentally sustainable approach. Studies on microorganisms as potential biocontrol agents or plant resistance inducers have given promising results. For example, bacterial symbionts that might be able to reduce phytoplasma transmission by leafhoppers have been identified. Reduced symptom expression in phytoplasma-infected plants treated with arbuscular mycorrhizal fungi, and the capacity of two fungal elicitins to prevent symptom expression in tobacco plants infected with stolbur phytoplasmas, were recently reported. In addition, the occurrence of mild strains of phytoplasmas might allow for disease control through cross protection. This WG will coordinate the results from epidemiological and molecular studies to formulate new and improved strategies for the control and management of phytoplasma diseases.

Task 1 will identify and/or breed varieties of crop plants that are resistant or tolerant to the phytoplasma/insect vector.

Task 2 will examine the effects of environmental factors on disease and symptom development.

Task 3 will integrate the results of tasks 1 and 2 into the best practices in disease control including prevention, eradication, control of vector, and best cropping systems.

Task 4 will provide data on the susceptibility of insect vectors to organic and low-impact insecticides in treatments schedules, especially at critical plant stages (e.g. pre-flowering and ripening) with the aim of safeguarding useful insects and avoiding pesticide residues in products.

Task 5 will identify endophyte populations in host plants and use knowledge of their interactions with both the host plant and phytoplasmas as tool for the establishment of an integrated, environmentally sustainable and innovative control of these diseases.

Task 6 will determine whether mild phytoplasma strains can effectively protect plants from infection by virulent (or severe) phytoplasma strains.

Working Group 4: Phytoplasma/host interactions

Over the past 5 years, European research teams have been involved in a number of phytoplasma full genome sequencing projects and some of this sequence information is available in public access databases. These projects have resulted in major advances in understanding phytoplasma genomics. The genomes themselves encode between 496 and 839 genes, and have very low G+C content (23-29.5 mol%). Whilst the main housekeeping genes appear to be conserved among phytoplasmas, there are also other genes that are unique to specific strains. Compared to other organisms, phytoplasmas lack genes encoding components of the pentose phosphate pathway, lack most genes for nucleotide synthesis, and also lack genes for the F0F1-type ATP synthase, which was previously thought to be a component of the minimal gene set required for all living organisms. Studies are currently identifying the various biosynthetic pathways that exist in phytoplasmas, and the transport mechanisms that are involved in importing essential compounds from host plants and insects, and in exporting potential pathogenicity factors into these hosts. In addition, there have been a number of studies to examine the changes in host gene expression that occur in infected plants, and the physiological and metabolic changes that occur in these hosts. Such studies have involved the use of differential display, cDNA-AFLP and microarrays technologies in a range of plant hosts, such as *Arabidopsis*, tomato, apple, pear, plum, *Catharanthus roseus* and poinsettia, and a number of up- and down-regulated plant genes have been identified in these different systems.

Task 1 will integrate all this genomics and bioinformatics information into a single website to allow password-protected access for all members who sign this Memorandum of Understanding. The content of the website will be modelled on similar websites, for example that of the European Arabidopsis Stock Centre at NASC Preparing annual reports.

Task 2 will aim to identify model systems in which to co-ordinate effort and research funding to enhance understanding of phytoplasma/host interactions. Ideally, such model systems should have a phytoplasma and a host plant for which the genome sequences are available, so that arrays can be used to examine changes in gene expression. However, other factors will be considered and discussed, such as the economic importance of the disease in Europe and knowledge of the phytoplasma/vector relationships. It is anticipated that through discussion and integration of knowledge, a consensus will be arrived at as to the most appropriate model systems on which to concentrate future efforts to maximize the impact of phytoplasma research aimed at developing novel means of disease control.

Task 3. Having available full sequence of a virulent strain of apple proliferation and of a second sequence of an avirulent strain of the same phytoplasma, it will be possible to explore the genome for differences and test individual candidate genes for their relevance in pathogenicity or host pathogen interaction by different technologies such as transgenic approaches and two yeast hybrids to devise possible practical use of these relevant information.

Task 4 aims to clarify aspects of population genetics of vector species and phytoplasma diversity by molecular typing of phytoplasmas and vectors with respect to their host plant affiliation. Basic understanding of the vector specificity of phytoplasmas and assessment of the risks of new phytoplasma-vector combinations leading to new or additional epidemic cycles branching to new crops will be achieved.

E. ORGANISATION

E.1 Coordination and organisation

The Action will be coordinated by a Management Committee (MC), which will consist of the Action Chairperson, Vice-Chairperson, the four WG Coordinators (see below) and the website/publicity manager. The MC will be charged with the following responsibilities:

- Appointment of the WG coordinators and of the website/publicity manager (Milestone MS1).
- Creating and maintaining the website to enhance communication among partners, disseminating results generated in the different WGs through Newsletter and fact-sheets, and enhancing public awareness (MS2).
- Planning and coordinating the different meetings and workshops, including MC meetings, scientific meetings, and a final symposium that will be organized to review the outcomes of the Action and to formulate plans for future networks and research collaborations (MS3).
- Promoting exchange of knowledge, data and collaborations between the partners from the different WGs.
- Establishing a programme for exchange visits between laboratories with special emphasis for early-stage researchers and scientists (Short term scientific exchanges, STSMs) from less developed regions of the COST countries (MS4).
- Assessment of the different activities (meetings, scientific exchanges, publications, training schools, website) to ensure that they meet the general objectives defined for the Action.
- Coordinating preparation of other EU founded proposals (e.g. Seventh framework proposals) that result from this Action, and dealing with any IPR matters that arise from the Action.
- Preparing annual reports (MS5).
- Coordinating and approving budget allocations.
- Meetings of the MC will take place once a year, normally linked to WG meetings or workshops. This will ensure efficient co-ordination of the activities and effective discussion of the objectives at critical points of the programme. The timetable for attaining the milestones is detailed in section F.

E.2 Working Groups

Four Working Groups (WG) will be established according to the four main tasks:

WG1: Detection/diagnostics; WG2: Epidemiology including vector ecology; WG3: Phytoplasma control in crop systems; WG4: Phytoplasma/host interactions.

Each of the WGs will be managed by a WG coordinator, and these coordinators will have the following main tasks:

- Participate in the Management Committee.
- Plan the details of the scientific meetings and training workshops within their WG.
- Co-ordinate the activities within their WGs to meet the objectives defined in the scientific programme.
- Promote the exchange of early stages researchers and materials among WG partners.
- Co-ordinate and manage the writing of reports for publication in the Newsletter, fact-sheets and on the website from their WG.
- Report the WG progress to the Action Chair and to the Management Committee.
- Meetings of the WGs will be organized on an annual basis at different partner locations. The Action will hold an annual 3-day meeting for each WG. The first two days of such meetings would allow exchange of specific information and ideas related to the WG, and encourage collaborations between scientists and institutes. The final day will involve the planning of exchanges of early-stages researchers for the following year, planning of a programme of training courses, and standardization of protocols, reports and materials for distribution to other WGs via the website and publications. This will enhance the exchange of materials and ideas between the different WGs.

E.3 Liaison and interaction with other research programmes

Advances in phytoplasma diagnostics achieved by WG1 will be presented in a joint seminar with EUPHRESCO to ensure rapid transfer of knowledge to European phytosanitary networks and major European agro-industries. Meetings will also take place throughout the Action between members of the MC and members of the other EU complementary programmes (see section B4), and the members will report back to the MC on progress in these programmes.

E.4 Gender balance and involvement of early-stage researchers

This COST Action will emphasize efforts to ensure a gender balance: in all its activities and in the Management Committee will place this as a standard item on all its agendas. Currently among the list of experts 50% are females.

The Action will also be committed to considerably involve early-stage researchers, in particular through the programme of exchange of scientific visits that will be organized in the different WGs but also planning special awards for the best research planned by early-stage researchers. The training and involvement of early-stage researchers will also be placed as a standard item on all MC agendas.

F. TIMETABLE

The Action will last for four years.

A kick-off meeting will start the Action, and during this meeting, the Action Chairperson, Vice-Chairperson, WG Coordinators and the website/publications manager will be appointed. - Milestone 1 (MS1).

The website homepage will be created immediately following this meeting and will be updated on a regular basis. – Milestone 2 (MS2).

A Newsletter will be published and distributed to members of the Action and to other interested parties on a regular basis.

Each WG will hold an annual meeting to discuss results and to disseminate these results to the rest of the Network. - Milestone 3 (MS3).

Each WG will aim to organize one training workshop and at least two scientific exchanges (STSMs) per annum. - Milestone 4 (MS4).

The Management Committee will meet annually, at one of the WG meetings or during a workshop, to prepare annual reports and review progress. - Milestone 5 (MS5).

A seminar will be organized by the MC during the 3rd year of the Action to promote and report the results to all the relevant agro-industry partners.

The Action will be closed with a final symposium, providing a meeting at which all the partners involved in the Action will be able to present their results.

	Year 1				Year 2				Year 3				Year 4			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
MC meeting	X				X				X				X			
WG meetings			X		X				X		X		X			
Inter WG meeting							X									X
WG1																
Development of diagnostic tools	X	X	X	X	X	X	X	X	X	X	X	X				
Validation of diagnostic tools											X	X	X	X		
Disseminate protocols															X	X
WG2																
Database for monitoring insect vectors	X	X	X	X												
Vector infectivity determination					X	X	X	X	X	X	X	X				
Other phytoplasma transmission ways					X	X	X	X	X	X	X	X	X	X	X	X
WG3																
Plant environment influence on diseases	X	X	X	X	X	X	X	X	X	X	X	X				
Best management practices									X	X	X	X	X	X	X	X
Role of endophytes in prevention	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
WG4																
Website genomic information	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Interactions in model-systems					X	X	X	X	X	X	X	X	X	X	X	X

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: Austria: AT, Belgium: BE, Bulgaria: BG, Croatia: HR, Czech Republic: CZ, Denmark: DK, Finland: FI, Former Yugoslav Republic of Macedonia: MK, France: FR, Germany: DE, Greece: GR, Hungary: HU, Israel: IL, Italy: IT, Lithuania: LT, Malta: MT, Netherlands: NL, Norway: NO, Poland: PL, Portugal: PT, Romania: RO, Serbia: RS, Slovenia: SI, Spain: ES, Switzerland: CH, Turkey: TR, United Kingdom: UK.

The above listed 27 COST countries, plus an institution from Bosnia and Herzegovina (BIH) have actively participated in the preparation of the Action or otherwise indicated their interest. It is anticipated that more participants will join the Action as they are made aware of its activities through the website. On the basis of national estimates, plus additional expenses that will be incurred for website administration and maintenance and Newsletter production, the economic dimension of the activities to be carried out under the Action has been estimated at 108 Million € for the total duration of the Action. This estimate is valid under the assumption that all the countries mentioned above but no other countries will participate in the Action. Any departure from this will change the total cost accordingly.

H. DISSEMINATION PLAN

H.1 Who?

The main target audiences for the dissemination of the results of the Action will be:

- other researchers from a range of phytoplasma-related disciplines, including pathologists, geneticists, diagnosticians, molecular biologists and entomologists, in both developed and developing regions within the COST countries.
- Members of the EUPHRESCO research framework involving representatives of phytosanitary administrations of 24 European countries.
- Crop-oriented national and international research and breeding institutes and companies.
- Government and regional agricultural policy makers.
- National Plant Health Inspectors and Quarantine Services.

- EPPO.
- Main agro-industries (farmers, nurseries and other manufactures and service providers).
- The general public.

H.2 What?

Dissemination methods:

- Press release following the kick-off meeting in all participating countries.
- Posting of general information on a public website.
- Posting of working documents on a password protected website.
- Technical bulletins from Working Groups, annual reports and final technical report.
- Publication of Proceedings from workshops.
- Guidelines and manuals for phytoplasma detection.
- Training events and workshops.
- Seminar organized by the MC for the agro-industry partners of the Action.
- Contributions to other national and international conferences and symposia.
- Articles in peer-reviewed scientific journals.
- Publications in national grower-oriented journals.

H.3 How?

Website

A public website will be established immediately after the kick-off meeting by the designated website/publications manager, and will be regularly updated. This will provide information to the international scientific community, among members of the Action, and to the wider interested bodies such as public officials and agro-industries and to the general public. Part of the website will be public access, whilst other information, for example non-published protocols and some bioinformatics information will be on password-protected webpages with access only to signatories of this Memorandum of Understanding. Contents of the website will be:

- General information about the COST Action and the meetings/workshops.
- Publications and contact details for Action participants.
- Teaching and research tools (protocols etc.).
- Links to phytoplasma genomics and other bioinformatics websites and to the websites of participating institutions.
- Trans-European vector monitoring information.
- Job announcements.
- Report of relevant results from short term scientific exchanges (STSMs) and of awards to early-stage researchers.
- Interactive public access section with details and pictures of phytoplasma diseases, allowing the public to report and send in pictures of suspected infected plants.

Publications:

- Scientific results of the project will be disseminated through refereed scientific journals provided that publication does not impair IPR.
- Newsletters, fact sheets and publications in non-scientific journals will help to disseminate the results to a broader, less specialized public.
- Technical articles and protocols will be produced for plant health inspectors and quarantine services.
- Proceedings of all meetings and workshops will be published and disseminated amongst members of the Action, and published on-line *via* the website.

Workshops and WG meetings:

The Action will organize workshops to inform regulatory bodies, growers etc. about new technologies developed through the project, and to provide hands-on training in techniques. These and the WG meetings will be organized in different countries throughout the four years of the Action to allow participants from all the COST countries to Network effectively, and to allow members from different states to appreciate the diverse phytoplasma-related issues throughout the COST countries.

International conferences:

Knowledge and data resulting from the Action will be presented at international conferences **to** disseminate the information to a wider scientific audience, including scientists from developing countries.

STMS:

Short-Term Scientific Missions (STMSs) will be offered to early-stage researchers, scientists and scientists from the less-developed European regions to facilitate technology transfer and to share protocols and new techniques.

Teaching activities:

Teaching activities in Universities at undergraduate and postgraduate level will take advantage of the Action, since many of the expected participants are from the University sector. Early-stage researchers will be exposed to the latest developments in phytoplasma research through this teaching, and enthused about this area of scientific research.