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WP4. Establishment of Diagnostic and Training Hubs (DTHs)

Prof. Dimitris Tsitsigiannis (Agricultural University of Athens-AUA)
and Prof. Magdalena Cara (Agricultural University of Tirana -AUT)

Networking to Improve Diagnostic Efficiency

Healthy Plants = Healthy World

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Description of WP 4

Diagnostic and training hubs (DTH) will be established at PIs in **partner countries** and equipped with additional **equipment** in order to serve as the **future regional centers of excellence** in the selected specific fields.

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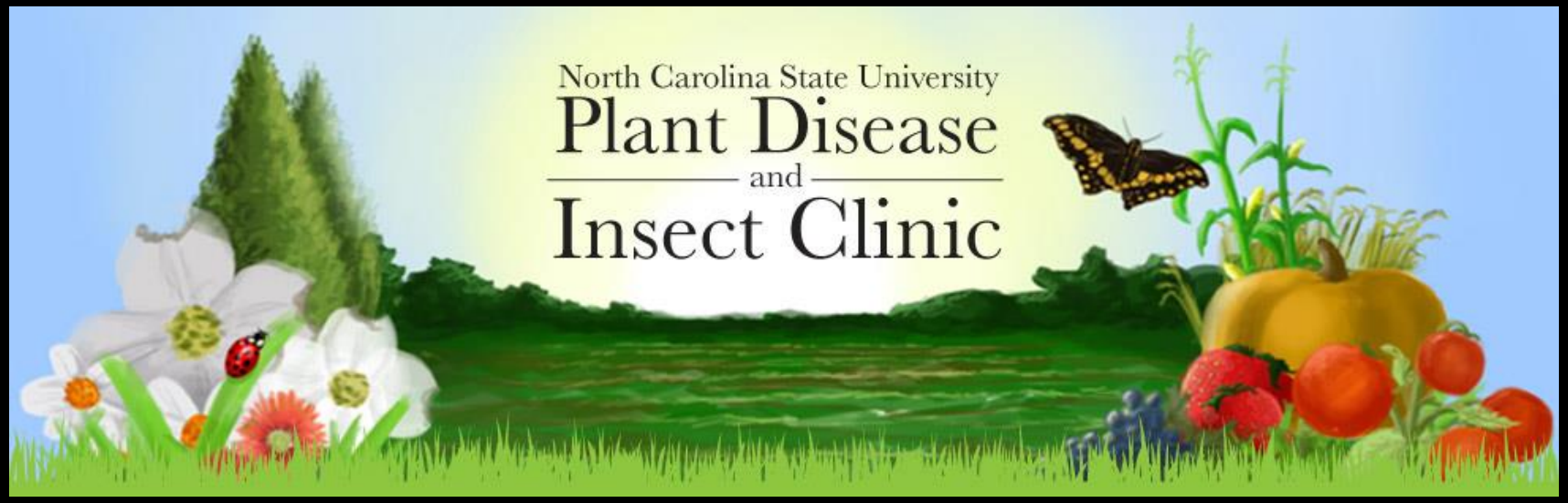
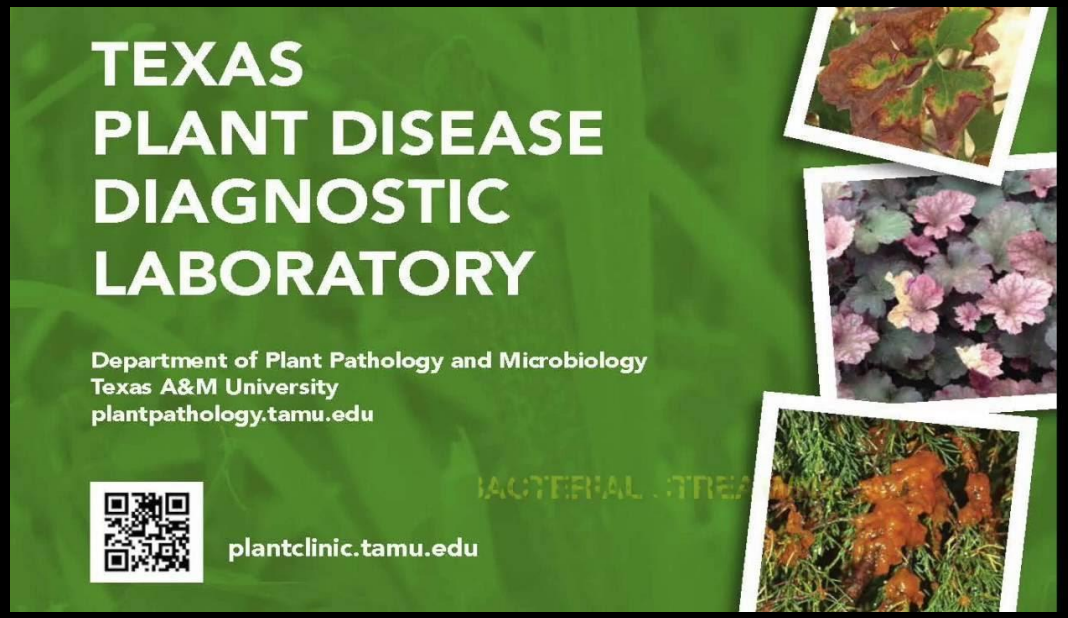
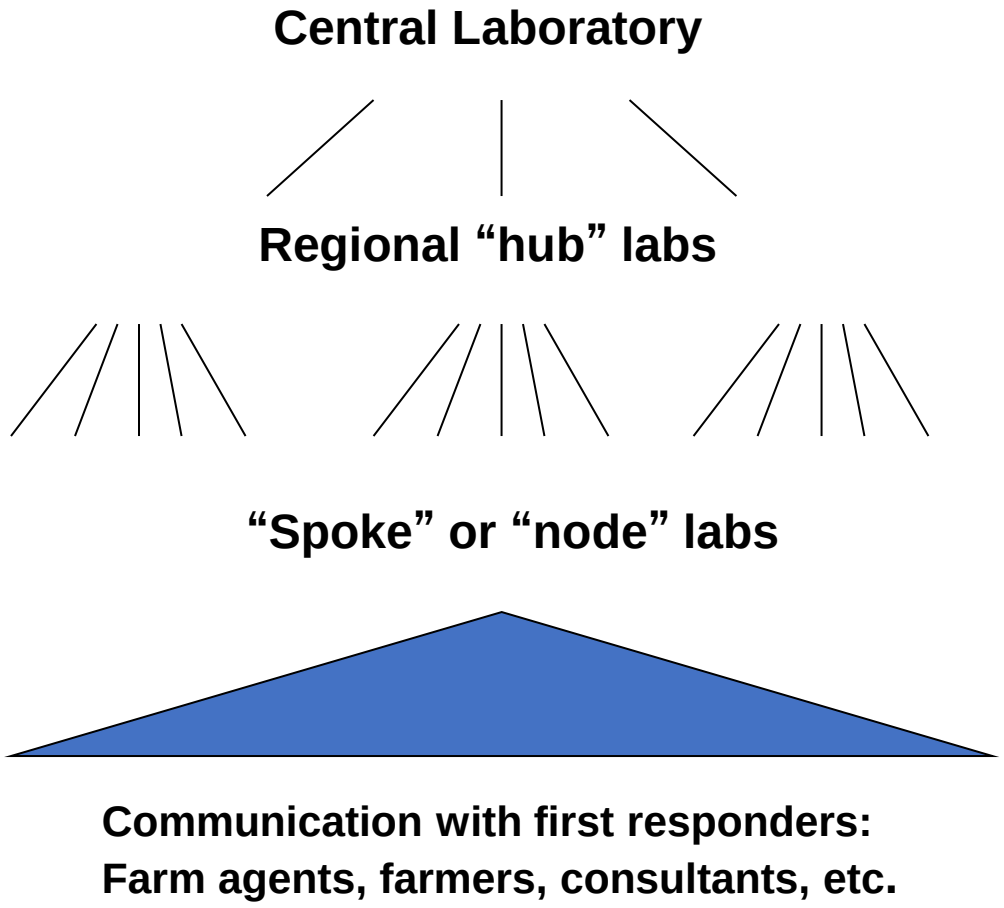

North Carolina State University
Plant Disease
and
Insect Clinic


University of Minnesota
Driven to Discover℠
Plant Disease Clinic


TEXAS
PLANT DISEASE
DIAGNOSTIC
LABORATORY
Department of Plant Pathology and Microbiology
Texas A&M University
plantpathology.tamu.edu
plantclinic.tamu.edu


Healthy
plants
healthy
planet
PLANT CLINIC
Jefferson County
Washington State University
Extension

Networking to Improve Diagnostic Efficiency (MODEL)



- Organized system of laboratories and personnel communicating with one another and working together
- Hierarchical structure
- Example: U.S. National Plant Diagnostic Network

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Objectives

- To develop the **selection criteria** and identify the excellence of scientific groups within the PIs from partner countries
- To **upgrade existing facilities** in service of PhD students', staff and professionals' needs
- To establish **diagnostic and training hubs** with high expertise in particular fields available to serve as regional centers for education and spreading knowledge

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Why to focus on plant disease diagnosis?

- **Plant diagnostics** has been called **an art and a science**
- The art of diagnosis is a **system of rules** or **governing principles** and implies a trained ability or mastery of science
- A good diagnostician is an agronomist with a **broad scientific knowledge** in subjects such as *plant pathology, entomology, botany, plant physiology, plant anatomy, soil science, cropping systems, horticulture, greenhouse/ nursery management, pesticides*
- **Successful diagnosticians** are **keen observers** and **good communicators**.

http://www.apsnet.org/publications/apsnetfeatures/pages/diagnostician.aspx

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Lettuce: *Sclerotinia, Pythium ή Verticillium*

Almond orchard

Phytophthora? Verticillium? Armillaria? Rosellinia?
Capnodis? Wood injury ? Water Logging ? ...

Olive: Nutrient Deficiency or Toxicity from weedcides, insecticides, fungicides, fertilizers?

Olives : *Colletotrichum, Alternaria, Fusarium or soft nose*

Grapevine: A particularly difficult case for diagnosis !!

Toxicity Diuron


Toxicity simazine and paraquat


Grapevine fanleaf virus




Viroid - grapevine yellow speckle


- Magnesium

Grapevine discoloration

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WP4 Tasks

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1. Selection criteria and evaluation procedure for diagnostic and training hubs

Selection criteria: regional distribution, experience and scientific excellence in related area, human capacities, existing and requested equipment, and strategic action plan of the diagnostic and training hub.

A template for the equipment list and criteria was developed

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PLEASE
DO NOT UNPLUG
ANY EQUIPMENT
ALONG THIS WALL.
THANK YOU

Disease Diagnostic Capacity in every partner

Component
Standard laboratory workspace
Microscopes - Stereoscopes
Laboratory supplies and consumables (media etc)
Specialized workspace for molecular diagnostics/PCR
Specialized equipment for molecular diagnostics/PCR
Specialized equipment for serological diagnostics
Growth Chambers - Greenhouse
Reference materials
Internet access - Computers
Cameras
???

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EQUIPMENT

List of existing equipment for each partner

- Refrigerators
- Autoclaves
- PCR
- etc …….

List of equipment that need to be purchased (34,000 € / partner country)

- microscopes
- Centrifuges
- entomological cages
- etc..

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2. Workshop: selection of candidates

WP4 members **discussed the equipment applications from partners**, proposed to **applicant improvements if needed** (regarding the type and performance of the proposed equipment) and selected the best candidates.

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3. Purchase of the equipment

For each institution, the list of the equipment approved during the selection process, the offers collected and the procurement process completed. The procurement were carried out according to the rules as proposed by EU commissions and local administration.

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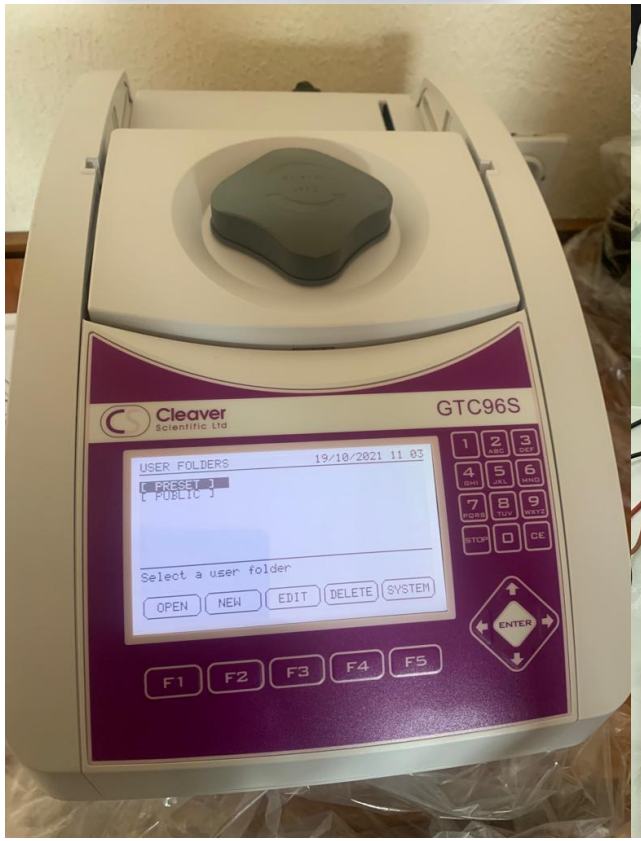
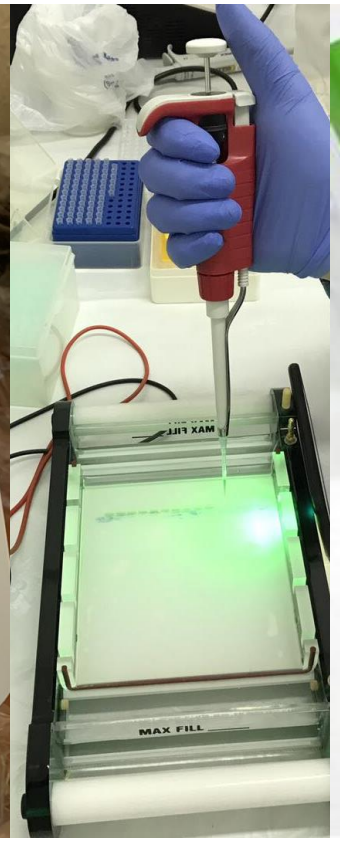
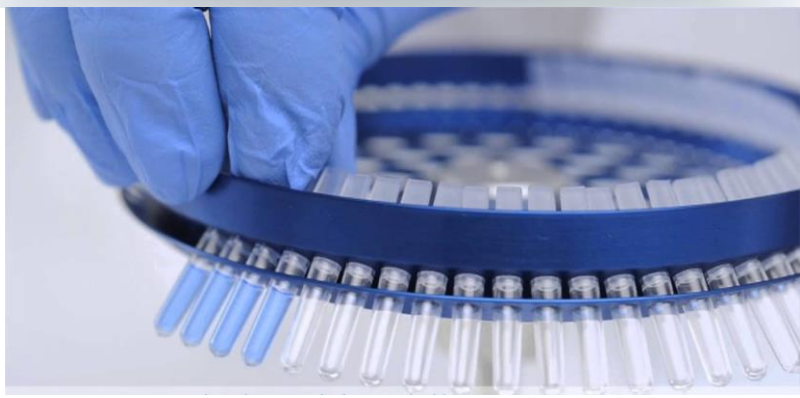
Estimated Start Date	**15.02.2019.**	**Estimated End Date**	**15.10.2022**
Lead Organisation	5. Agricultural University of Athens (AUA) 6. Agricultural University Tirana (AUT)		
Participating Organisation	1. University of Zagreb Faculty of Agriculture (FAZ) 2. University of Osijek (PFOS) 3. University of Aldo Moro Bari (UNIBA) 4. Agricultural University Plovdiv (AU) 7. University F.S. Noli Korce (UNKO) 8. University of Sarajevo (UNSA) 9. University of Mostar (SVEMO) 10. University of Belgrade (UB) 11. University of Novi Sad (UNS) 12. Biotechnical University of Montenegro (UOM)		

New equipment







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Agricultural University Tirana

Real – Time PCR

Vortex

Balance

0.2 ml PCR strip tube
small Centrifuge

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University F.S. Noli Korce

Insect net cages and small incubators

Analytical Balance

pH meter

Centrifuge

Gel electrophoresis

Microscope

Incubator

Vortex

Liquid Nitrogen Container

Thermocycler

Gel reader

Refrigerator

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University of Mostar

Fluorescence Microscope

Microscope

Desktop computer

Autoclave

Nozzle Tester

PCR Thermal cycler

Electrophoresis and vortex

Insect handling cage

Insect collecting equipment (aspirators, sweep net, beating sheet)

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University of Belgrade

LAMP apparatus

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University of Belgrade

Desktop Computers

Laboratory pesticide spraying chamber

Ice producing machine

Stereoscopes with camera

Refrigerator

Digital drawing table

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University of Novi Sad

Insect rearing chamber

Ultra freezer

Giga-8dd Basic 8 channel EPG recording system

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University of Montenegro

Gel documentation

PCR Thermal Cycler

Growth chamber

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4. Forming a network of DTHs, signing agreements between DTHs

Establishment of a network of diagnostic laboratories

Diagnostic and Training Hubs

This network is led by the Agricultural University of Tirana (AUT) and supported by universities of EU countries

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Mutual cooperation agreement between laboratories as a HarISA product

UNIVERSITY OF ZAGREB
FACULTY OF AGRICULTURE
Address
Country

Concludes an

AGREEMENT OF SCIENTIFIC COOPERATION

University of Zagreb Faculty of Agriculture (FAZ), Josip Juraj Strossmayer University of Osijek (FAZOS), University of Aldo Moro Bari (UNIBA), Agricultural University Plovdiv (AU), Agricultural University of Athens (AUA), Agricultural University Tirana (AUT), University F.S. Noli Korce (UNKO), University of Sarajevo (UNSA), University of Mostar (SVEMO), University of Belgrade (UB), University of Novi Sad (UNS) and University of Montenegro Biotechnical Faculty (UoM) (hereinafter referred to as „Parties") wishing to promote scientific, academic and educational cooperation among the Institutions, in the terms stated henceforth, have concluded the present Agreement of Scientific Cooperation.

This Agreement aims to faciliate a general framework for facilitating institutional collaboration and cooperation between the Parties in the area of establishment of the Diagnostic and Training Hubs (DTHs). The core of DTHs is the plant protection laboratories, which are already equipped with infrastructure suitable for the identification of plant pathogens/pests by advanced molecular methods, in the framework of Erasmus+ CBHE - HarISA project. DTHs will be established at PIs in partner countries and equipped with additional equipment in order to serve as the future regional centers of excellence in the selected specific fields.

II Subject of the Agreement

Cooperation will be implemented through, but may not be limited to, the following activities:

- to identify capacity development of diagnostic laboratories of partner universities
- to establish Diagnostic and Training Hubs with high expertise in particular fields available to serve as regional centers for education and spreading of knowledge
- to establish a network of diagnostic laboratories of partner universities
- to foster networking to improve diagnostic efficiency in an organized system of laboratories and personnel communicating with one another and working together
- to introduce distance diagnostics and data management web portal
- to harmonize diagnostic protocols (Standard Operating Procedures - SOPs) to ensure that each laboratory will carry out the same way plant pest diagnosis by clearly defining and documenting the procedures and processes that are to be used to complete the task. SOPs are task-specific documents that describe clearly all actions that are required to complete the task clearly, concisely and completely and help in achieving and maintaining high levels of accuracy and repeatability of experimental results regardless of changes in personnel or equipment or other operating conditions used.
- to provide a system of diagnostic rules or governing principles
- to develop and test diagnostic assays
- to carry out joint diagnoses and publications with harmonized protocols
- to introduce inter-calibrations between laboratories and proficiency tests that are mandatory in future accreditations of plant diagnostics clinics
- to foster networking and mutual exchange of academic staff, research personnel, students, and other associates for lectures, visits, and transfer of knowledge
- to promote and conduct joint educational, training programs and research activities in plant disease diagnosis such as educational programs of doctoral and postdoctoral studies and projects
- to promore joint participation and applying for funds designated for research and education in plant disease diagnosis
- to jointly organize conferences, symposia, congresses, seminars, courses, summer schools, workshops, and other meetings in plant disease diagnosis
- to exchange professional literature, textbooks, and other university publications.

In the implementation of specific cooperative programs, a writen agreement covering all relevant aspects including funding and other obligations to be undertaken by each party will be negotiated, mutually agreed and formalized in writing, prior to the commencement of the program.

III Duration of the Agreement

This Agreement will become effective upon the date of signature of both institutions. It will be in force for five (5) year and may be renewed by the Parties for further period.

Amendments and changes will be possible only in case of written consent by all Parties and will be added to this Agreement.

This Agreement may be terminated before the expirary of the five (5) year period only upon written consent of all Parties. Either Party wishing to make changes or terminate the Agreement shall notify all other institutions in writing of such intention no later than six (6) months before the suggested changes or termination should come into effect. The Agreement cannot be terminated if this action will jeopardize the implementation of any of the agreed activities, or before the deadline set for completion of such activities.

IV Implementation of the Agreement

Each institution will appoint a contact person to coordinate the implementation of this Agreement. Both Parties have committed not to execute activities related to this Agreement without previous consultations.

All Parties agree that all financial agreements necessary to implement this Agreement must be negotiated and will depend upon availability of funds. Neither institution shall have any financial obligation to the other institution based on this Agreement.

In witness whereof, the parties here to affix their signatures below this

For University of Zagreb
Faculty of Agriculture (FAZ)

Prof. dr. .....

Date: Date:

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Contact: harissa@agr.hr
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Subject of the Agreement

- to establish Diagnostic and Training Hubs with high expertise in particular fields available to serve as **regional centers for education and spreading of knowledge**
- to establish a **network of diagnostic laboratories** of partner universities
- to **foster networking** to improve diagnostic efficiency in an organized system of laboratories and personnel communicating with one another and working together
- to introduce **distance diagnostics** and data management web portal

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Contact: harissa@agr.hr
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Subject of the Agreement

- to **harmonize diagnostic protocols (Standard Operating Procedures - SOPs)** to ensure that each laboratory will carry out the same way plant pest diagnosis by clearly defining and documenting the procedures and processes that are to be used to complete the task.
- to provide a **system of diagnostic rules** or **governing principles**
- to **develop and test diagnostic assays**
- to carry out **joint diagnoses** and **publications with harmonized protocols**
- to introduce **inter-calibrations between laboratories** and proficiency tests that are mandatory in future accreditations of plant diagnostics clinics

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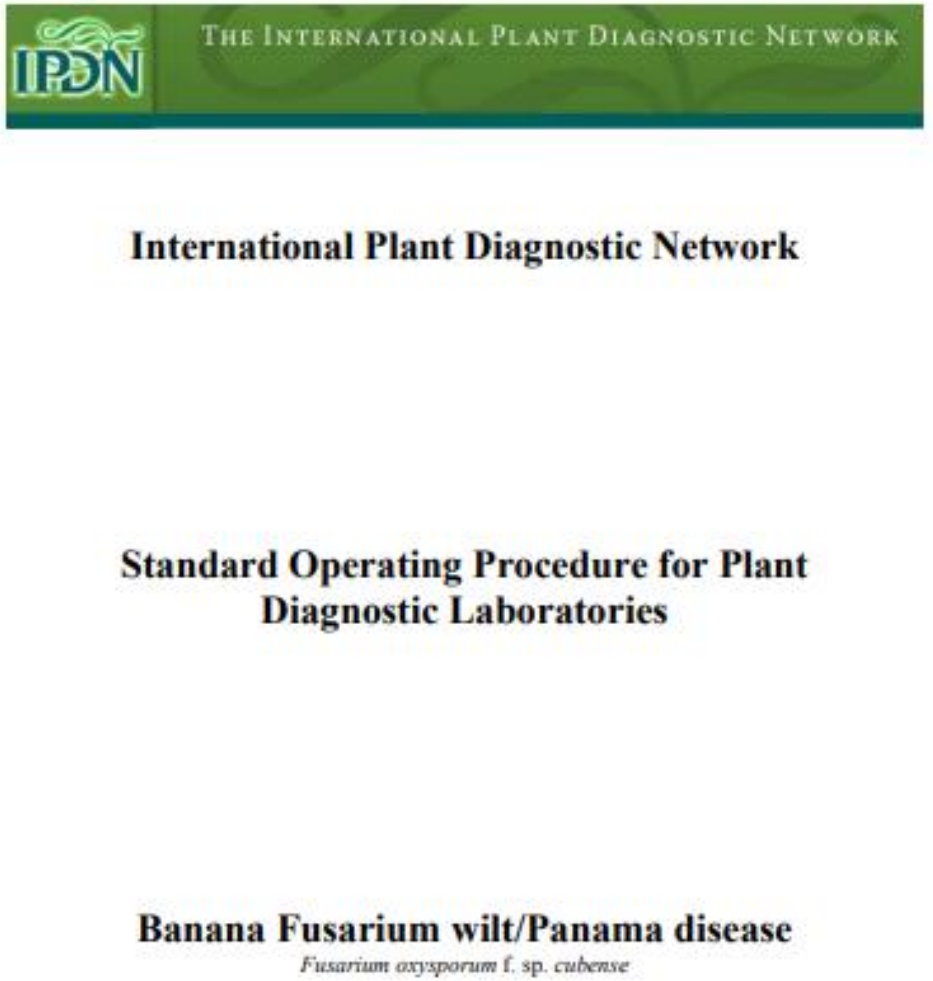
Subject of the Agreement

- to **foster networking and mutual exchange of academic staff, research personnel, students, and other associates** for lectures, visits, and transfer of knowledge
- to **promote and conduct joint educational, training programs and research activities** in plant disease diagnosis such as educational programs of doctoral and postdoctoral studies and projects
- to promore joint participation and **applying for funds designated for research and education in plant disease diagnosis**
- to **jointly organize conferences**, symposia, congresses, seminars, courses, summer schools, workshops, and other meetings in plant disease diagnosis
- to exchange professional literature, textbooks, and other university publications.

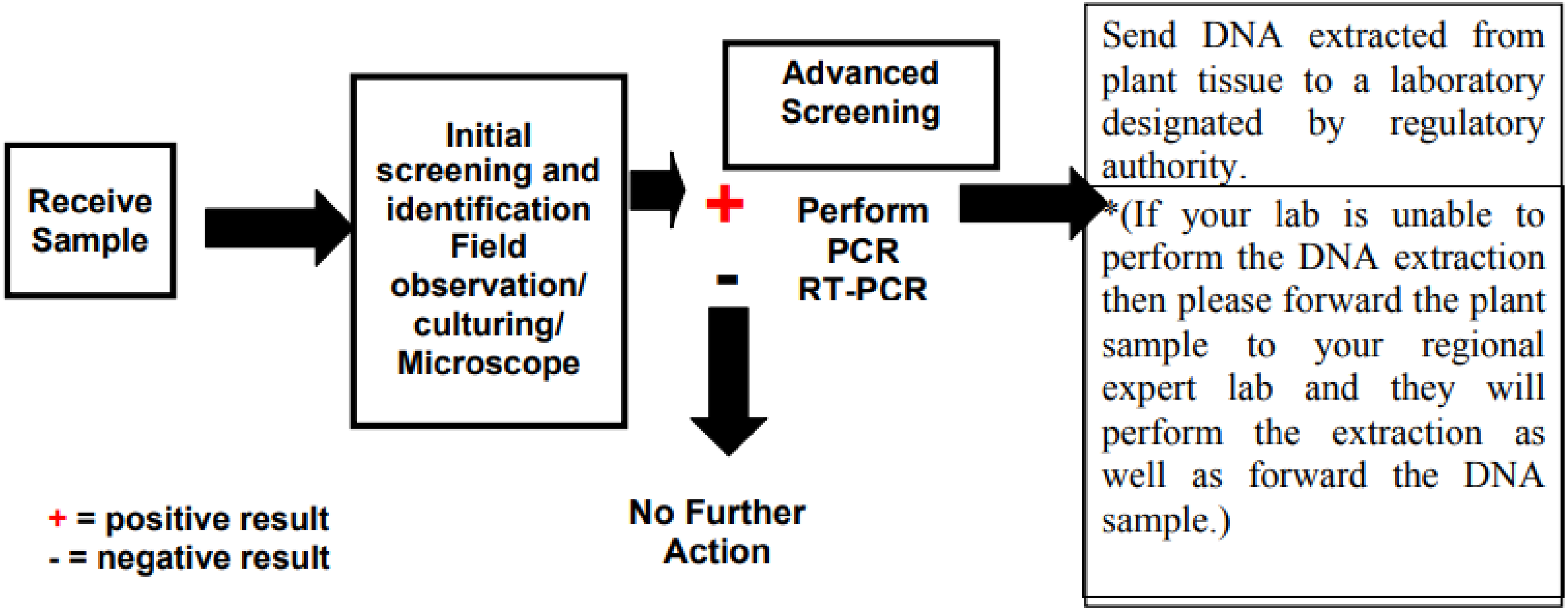
Standard Operating Procedures (SOPs)

- Background
- Sampling
- Symptoms & Signs
- Media recipes
- Morphological caracterisation
- Serological & Biochemical tests
- DNA/RNA extraction
- PCR protocols & identification
- Sequencing & Phylogenetic analysis
- Widely tested and validated



http://www.sun.ac.za/english/faculty/agri/plant-pathology/ac4tr4/Documents/Revised%20SOP_Panama_IPDN.pdf

Disease Sample Protocol Flowchart



http://www.sun.ac.za/english/faculty/agri/plant-pathology/ac4tr4/Documents/Revised%20SOP_Panama_IPDN.pdf

Sampling preparation for ELISA, DTBIA, PCR and LAMP

+ 5-8 ml
buffer (1:10)

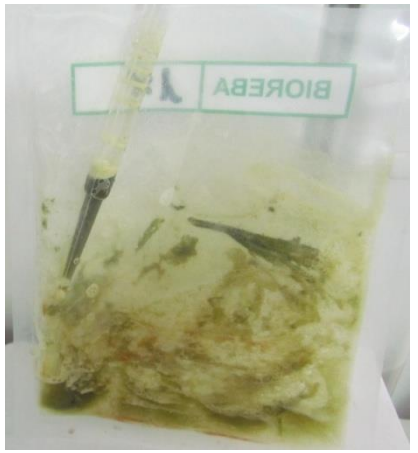
Fig. 5 Tissue extracts for ELISA test in DPP laboratory.

0.5-0.8 gr inde te mostres perfaqesuese

Liquid-nitrogen

Process 100 mg

DNA extraction or
ELISA/LAMP sap preparation

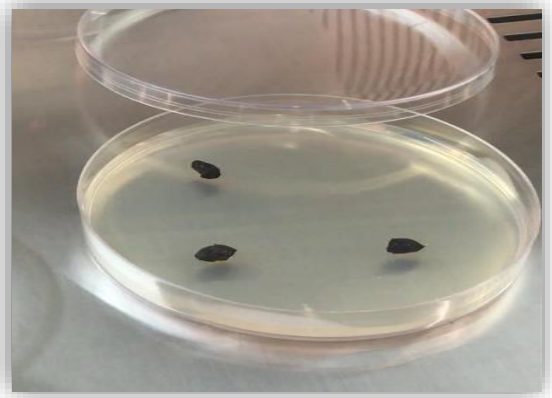


1 ml crude sap

ISOLATION PROCEDURES

1. External lesions and/or slight internal rot

Surface sterilization by NaOCl (2%) for 2 min, rinsed for 1 min by sterile distilled water and air-dried in sterile conditions



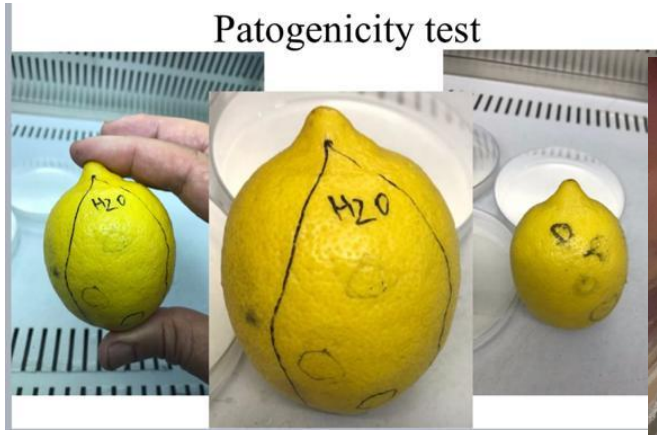
2. Severe and liquid rot

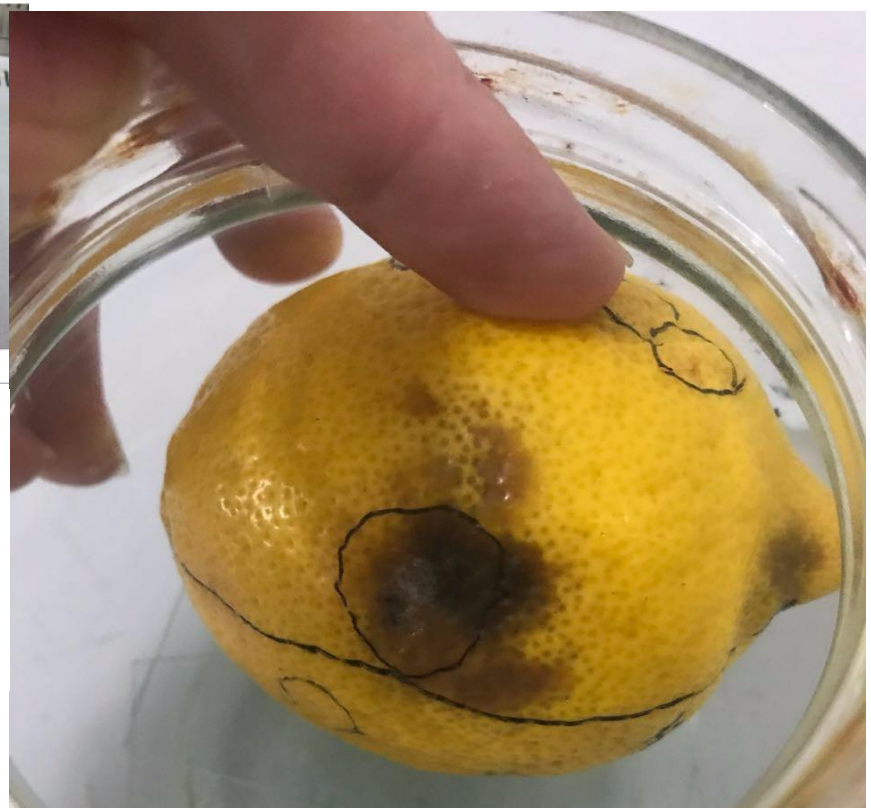
Sterilization by spraying a 70-90% ethanol solution followed by air-drying in sterile conditions

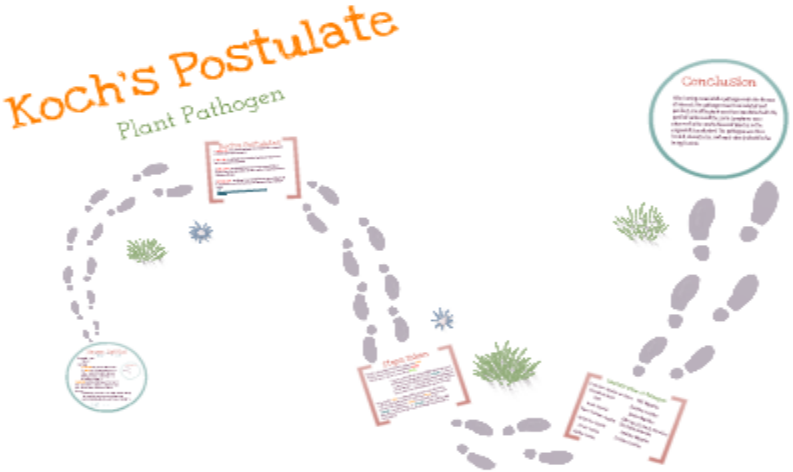
Small tissue portions plated on semi-selective Potato Dextrose Agar (PDA) amended with ampicillin and streptomycin (250 mg/L each)

(Mincuzzi *et al*., 2020; Garganese et al., 2016)

Patogenicity tests

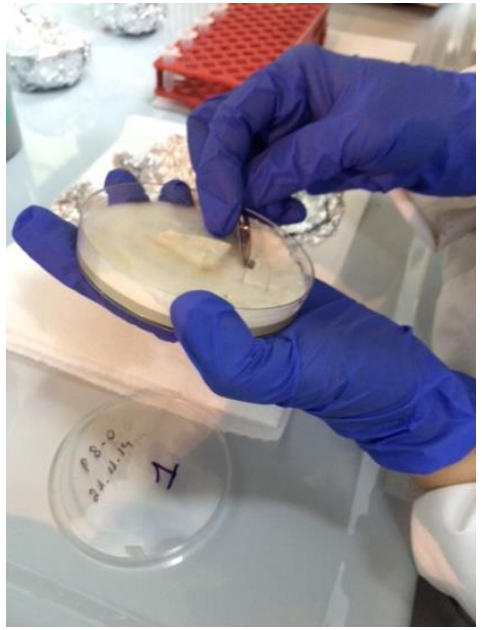


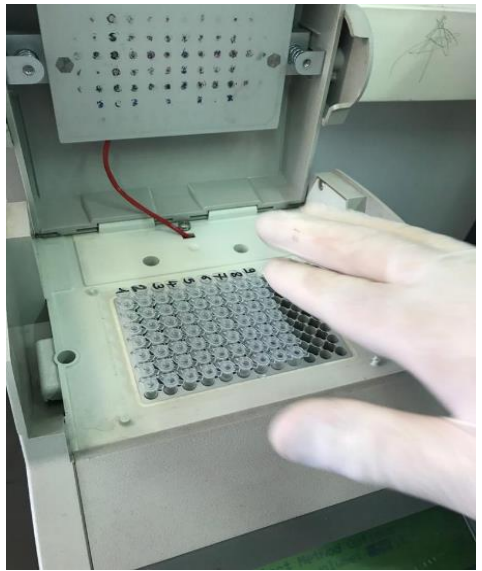


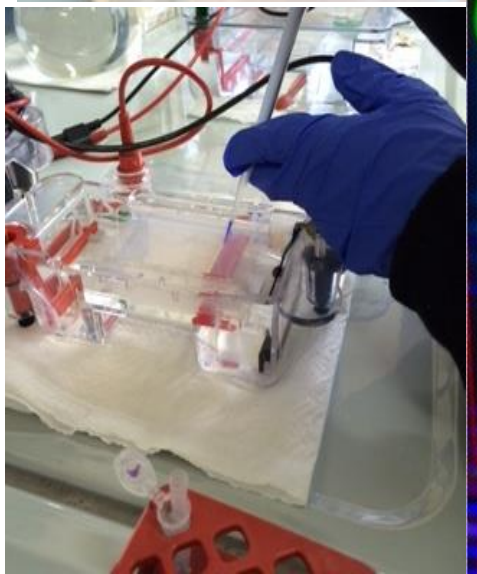


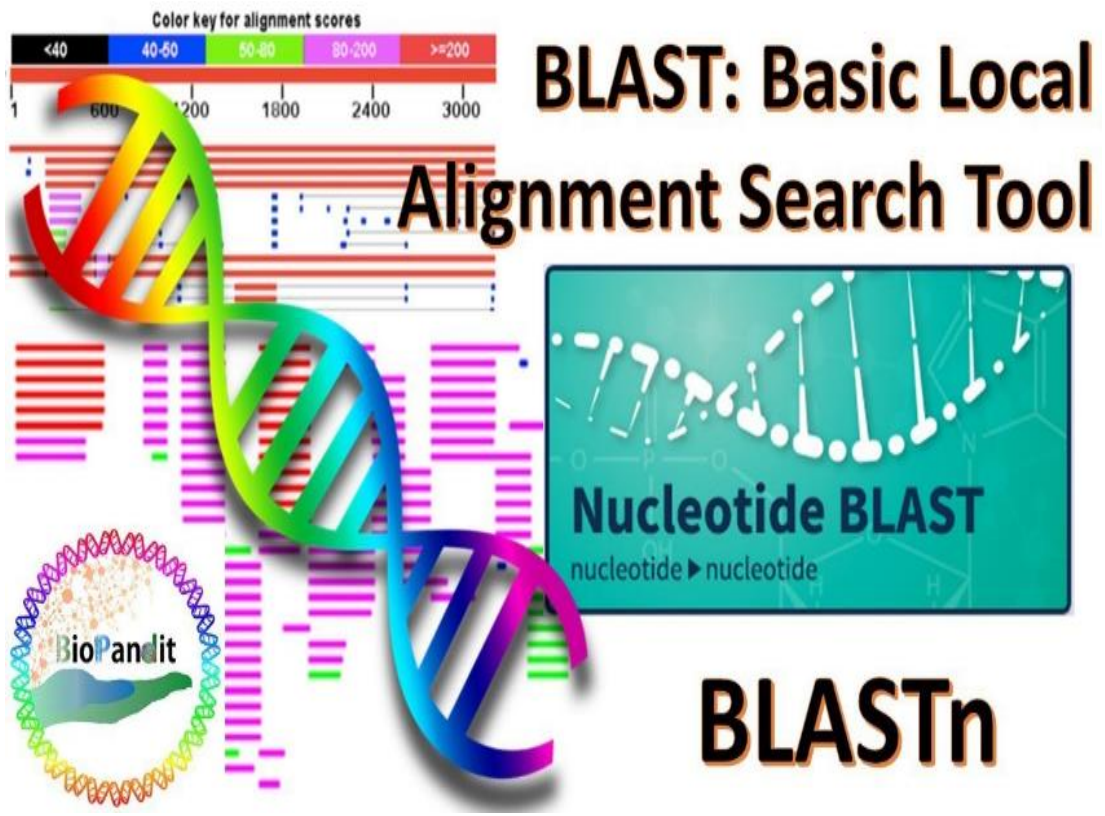
Colletotrichum gloeosporioides

DNA/RNA extraction, PCR, Sequencings









BLAST sequence analysis (accession KY751715) at GenBank revealed 99% similarity with *Phytophthora capsici* AJ854285.

Inter-calibrations between laboratories and proficiency tests

- 1st International external quality assessment studies for laboratory diagnosis of ***Xylella fastidiosa***. (35 Laboratories).
- Intercalibrations have been performed between AUT and the University of Bari and CIHEAM for some plant pathogens. (*Colletotricum gloesporoides, Rhizopus arizus, Alternaria alternata,* etc.
- Intercalibrations with the University of Catania for *Colletotricum gloesporoides* in citrus.
- Intercalibrations with the Agricultural University of Athens for *Colletotrichum acutatum* in olives.

Carrying out joint plant disease diagnoses and publication with harmonized protocols

Research article

Received: 18 December 2017 Revised: 13 June 2018 Accepted: 17 June 2018 Published online in Wiley Online Library: 9 September 2018

(wileyonlinelibrary.com) DOI 10.1002/jib.521

Isolation of *Rhizopus arrhizus* from Albanian barley

Magdalena Cara,[1] Simona M. Sanzani,[2]* Annamaria Mincuzzi,[2] Antonio Ippolito,[2] Orges Cara[1] and Jordan Merkuri[3]

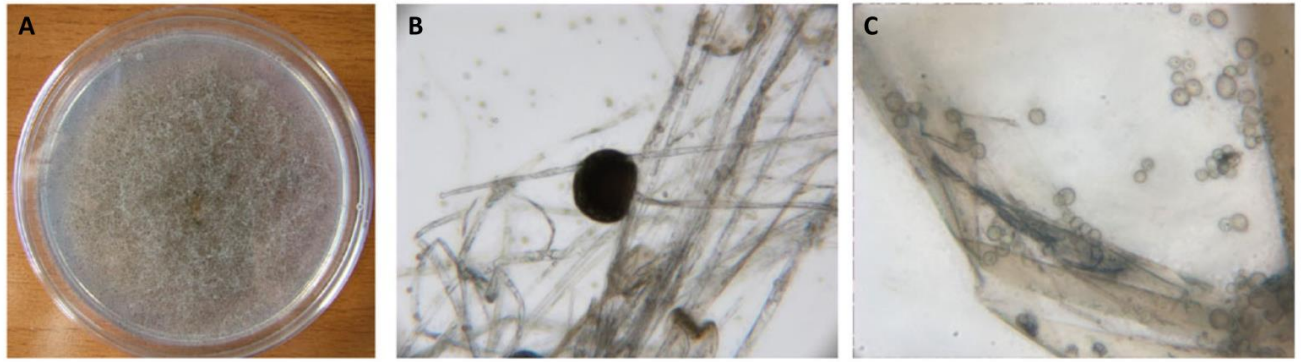


Figure 1. Macro- and microscopic characteristics of *Rhizopus arrhizus* on PDA at 25°C in the dark. [Colour figure can be viewed at wileyonlinelibrary.com]

sustainability

MDPI

Article

Optical Characterization of *Alternaria* spp. Contaminated Wheat Grain and Its Influence in Early Broilers Nutrition on Oxidative Stress

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check for updates

plant disease

First Report of *Colletotrichum acutatum* Causing Anthracnose on Olives in Albania

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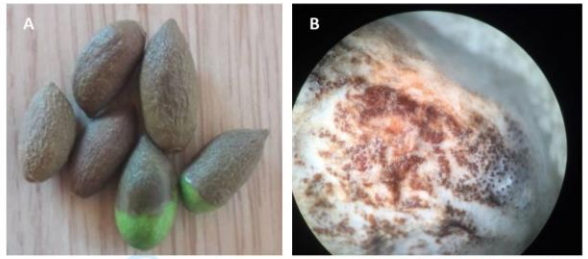

Figure S1. Symptoms of anthracnose on olive fruits and morphology of the *Colletotrichum acutatum*.

Symptoms of mummified olive fruits (A), acervuli of *C. acutatum* on olive fruits after artificial infection

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Short communication

Colletotrichum gloeosporioides sensu stricto as causal agent of anthracnose on pomegranate fruit in Albania

Magdalena Cara [a], Annamaria Mincuzzi [b], Jordan Merkuri [c], Hekuran Vrapi [a], Orges Cara [a], Antonio Ippolito [b], Riccardo Baroncelli [d], Simona Marianna Sanzani [e,*]

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First Report of Phytophthora Blight of Cucurbit Caused by *Phytophthora capsici* in Albania

M. Cara, T. Yaseen, and J. Merkuri

Affiliations

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Co-funded by the European Union

Preliminary results of DTHs

Sequencing and registration in repositories of economically relevant pathogens

- *Colletotricum gloeosporioides* in citrus and pomegranate,
- *Phytophthora nicotianae* and *P. citrophthora* in citrus,
- *Colletotricum acutatum* in olive,
- *Phytophthora capsici* in cucumber, *etc*.
- More than 70 sequences were registered in GenBank.
- 32 strains of Alternaria alternata are already submitted in ncbi
- These pathogens are stored in standard cultural collections at the AUT and in partner universities.

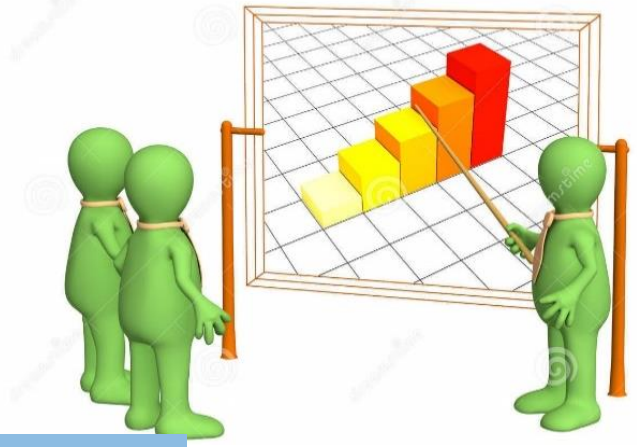
Harmonization and Innovation in PhD Study Programs for Plant Health in Sustainable Agriculture –HarISA is a Erasmus+ project Co-funded with the suport of the European Union. Project Number: 598444-EPP-1-2018-1-HR-EPPKA2-CBHE-JP (2018-2472 / 001-001)

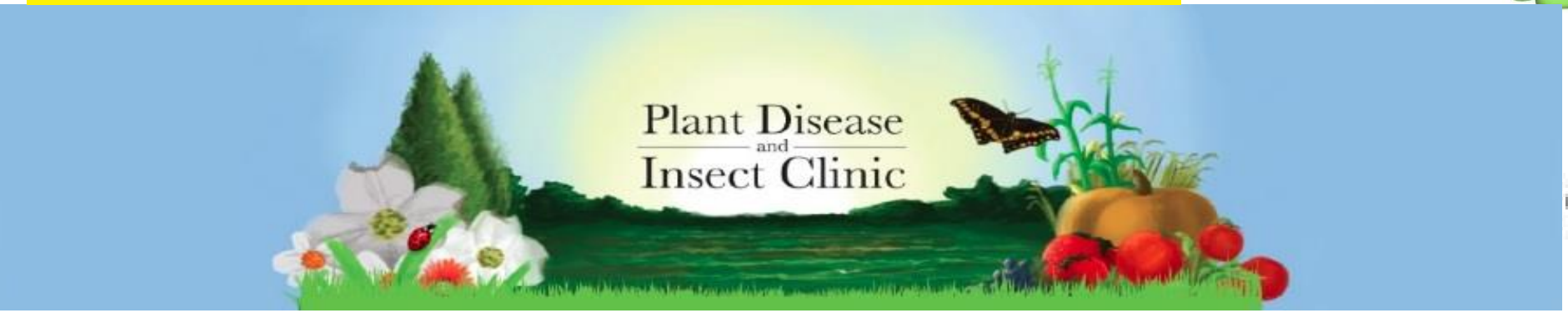
Preparation of training program for Ph.D. Students and young researches

2020

Manual on

PLANT HEALTH CLINIC





training hubs

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Expanding the network with partners interested in cooperation

- Dept. Agriculture, Food and Environment, University of Catania (ERASMUS ICM)
- CIHEAM, Bari (Master thesis supervisor)
- Plant Pathology Lab, Faculty of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki (ERASMUS ICM applic.)
- University of the West of Scotland – (ERASMUS ICM)
- CNR – Bari Italy (Bilateral Project)

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Projects that support DTHs

- Horizon Europe – focus for all researchers
- ERASMUS PLUS CBHE & ICM
- Jean Monnet (Module, chair, network, excellence center)
- COST Action (excellent networks, COST Inclusiveness Target Countries (ITCs)
- Maria Sklodowska Curie – Ph.D. students
- National and Bilateral projects – Ph.D. students

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