Project Title:

A MICROARRAY TOOL FOR EFFECTIVE CONTROL OF FUNGAL GRAPEVINE TRUNK DISEASES IN EUROPEAN VINEYARDS

Project Acronym: MYCORRAY

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Final Report
Publishable Summary

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Executive Summary

MYCORRAY was a project funded by the European Commission´s Seventh Framework Programme (FP7) under the “Research for SMEs” Capacities Programme with the aim of developing a cost-effective, accurate, reliable diagnostic tool for early and simultaneous identification of the 13 main fungi involved in grapevine trunk diseases. The project started in February 2013 and had a duration of 30 months. The 1.1 million Euros contribution from the European Commission allowed bringing together European technology companies, research institutes, universities and technological centres, as well as farms and wineries, from six different countries in Europe: France, Italy, UK, Spain, The Netherlands and Hungary.

The main result of the project was a disposable array tube containing a DNA microarray chip and a cost-effective standalone array reader suitable for use by non-specialists. The microarray chip has been designed to detect 12 of the most important fungal species involved in grapevine trunk disease. In addition, the array can be used to detect the presence of the bacterium *Agrobacterium vitis* and distinguish between strains harbouring the Ti plasmid. The array is highly specific allowing sensitive and accurate detection of grapevine pathogens and can reduce the time required to obtain results. The reader is an Android-based and fully-customized device that captures microarray chip images, process them in order to detect the presence of targeted pathogens and delivers reports to the user.

The MYCORRAY system was validated not only on a laboratory scale with cultures to assess specificity and the limit of detection for each pathogen, but also using naturally infected grape vine samples and comparing with results from three different detection methods. As a conclusion, the MYCORRAY system can detect 13 pathogens simultaneously using a cost effective, standalone user friendly scanner, with the results displayed in a format that is easy to interpret. The MYCORRAY system is specific and fit for purpose; it can detect pathogens in grape vine material collected from across Europe. The MYCORRAY workflow is simpler than performing numerous separate real time PCR assays or nested PCRs for a single sample to determine the same information. Furthermore the approach of nested PCR requires samples to be subjected to DNA sequencing to confirm the exact organism present; this is both time consuming and expensive. There are not real time PCR and nested PCR assays for all 13 of the target pathogens, giving the MYCORRAY system an advantage in its ability to detect a wide range of pathogens in a single test.

All partners contributed in dissemination of MYCORRAY project and participated in a wide variety of actions during all the project’s lifetime including workshops, exhibitions, conferences, presentations, courses, fairs and interviews. During each of them, the partners that attended used one of the MYCORRAY prototypes to show the technology and how the tests are done.

The project ended in July 2015 and results were promising. Following the completion of the project, MYCORRAY system was planned to be further developed into a marketable product by a group of partners who will bring it to the market in the shortest possible time.

Summary description of project context and objectives

45% of world's 225 million hectares of grapevines are grown in Europe, the global lead producer and exporter of wine. Italy, France and Spain are the main producers accounting for one third of total world production of 66 million Mt. The European Union is also world leader in the market for vine nursery products, with an annual output of 360 million cuttings. The competitiveness of European wine market SMEs, already facing growing international competition from New World and Asian producers, is currently severely threatened by complexes of grapevine trunk wood fungi, responsible for the most destructive diseases of grapevines namely Eutypa dieback, Esca disease, Botryosphaeria canker and dieback (or Black Dead Arm), and Black Foot disease.

Already widespread in most vine-growing regions of France, Spain, Italy, Portugal, Switzerland, Austria, Germany, Slovakia, Hungary, Greece, Romania and beyond the borders of the EU, the incidence of these diseases and the frequency and severity of their symptoms has drastically increased over the last decade. Despite governmental and research efforts, to date no single effective treatment to fight these diseases has been proposed to winegrowers, meaning that efficient control requires an Integrated Pest Management Strategy based on preventive measures. Available measures include the removal and destruction of infected vines or trunk parts to minimize the possible infection sources, the protection of the pruning wounds with fungicides (e.g. tebuconazole, cryptonol), cultural practices such as late pruning and the use of pathogen-free planting material. Visual inspection remains routine practice to determine the presence of infections. However, grapevine trunk diseases are very complex, as they usually involve more than one causal organism and can coexist in the same vine or evolve into each other depending on external factors. The different symptoms, often similar between the different diseases but very variable in different cultivars, make visual diagnostics extremely unreliable, while other available methods only detect one organism per test. In the case of grapevine trunk diseases, which require at least eleven tests per vine (one per pathogen), the cost (€60 per test) and logistical problems associated to parallel testing make the detection of all the 13 pathogens impractical and prohibitively expensive, and ultimately the control measures not effective, as most small and medium winegrowers will test for only some but not all pathogens. Diagnostics must strive to mirror the true complexity of the fungal trunk disease reality, and provide European winegrowers and vine nursery SMEs with a tool like MYCORRAY that allows the simultaneous cost-effective testing of the most relevant fungi and that gives information on the phytosanitary status of their vineyards and nursery plantations so that they can adapt management practices and perform immediate, precise and efficient interventions to control these pathogens, their active spread and associated losses. The elimination only of confirmed infected vines will result in considerable savings for European winegrower SMEs, while treatments based on actual pathogen populations will increase the efficiency while reducing costs and environmental impact of indiscriminate fungicide use for improved sustainable agriculture practices.

Driving consortium SME end user partners are certain that the development of a cost-effective, accurate, reliable diagnostic tool for early and simultaneous identification of the 13 main fungi involved in grapevine trunk diseases is a clearly needed first step in the successful integrated management to limit their crippling impact. The cost of the developed tool will be below €2,000 per unit and will be suited to use in end-user´s routine testing laboratories.

Description of the main scientific and technological results and foregrounds

MYCORRAY was developed by a consortium of organizations including companies in the vineyard and wine industry, technological companies, universities, research institutes and technological centres. The project partners included: the grapewine nursery Mercier Frères (France), the farm and winery Antonelli San Marco (Italy), the large winery Torres (Spain), the phytodiagnostics company SEDIAG (France), the manufacturer of rapid diagnostic solutions Forsite Diagnostics (UK) and the technology company JCB Electromecánica (Spain). The R&D and demonstration activities were performed by the R&D company Ateknea Solutions (Spain), the Food and Environment Research Agency FERA (UK), the University of Florence (Italy) and the Agricultural Research Institute of the Wageningen University (The Netherlands).

Working together since 2013, the cooperation between these organizations has led to the following scientific and technological results, which are summarized below:

Main technological result: the MYCORRAY reader

MYCORRAY reader is an Android-based and fully-customized device to process micro-array tubes using advanced processing image algorithms in order to detect several pathogens in vineyards. There is not any device currently in the market capable to detect more than one fungi at once but MYCORRAY is capable to detect several diseases or fungi infections just running one simple analysis in less time and lower price than competitors. The reader consists of three main parts: optical unit, processing unit and software application.

Figure 1. MYCORRAY reader and application

Optical unit

For capturing the microarray and amplifying the image, MYCORRAY integrates a low-cost, highly sensitive vertical optical system which consists in three main components: camera, illumination and zoom lenses. The camera used for the acquisition of the microarray images contains a CMOS ¼ inch image sensor and provides VGA image resolution, auto-white balance, auto-brightness and auto-contrast. The image is illuminated using a white LED flash. Optical amplification is realized through a zoom lenses system.

Processing unit

The BeagleBone Black is selected to be the main controller of the system. This has been assembled on a custom PCB with several inputs and outputs and peripheral components in order to build a robust and powerful device demonstrating quick response and high performance.

The main features of this unit are listed below:

- Android 4.2 Jelly Bean

Sleek, smart and full of innovative features, the MYCORRAY reader is equipped with Google Android 4.2 "Jelly Bean" operating system, which features improved multi-tasking performance, more reactive touch responses, offering a smoother and more consistent rendering across the system through vsync and triple buffering.

- Powerful core, fast memory and SSD storage

The core of the processing unit features a Sitara™ ARM® Cortex-A8 processor from Texas Instruments @ 1GHz along with 512MB of fast DDR3L RAM memory, 4GB eMMC flash storage and an internal memory of 8GB microSD.

- 7-inches Multi-Touch Display

Great accuracy 7" capacitive touchscreen display with a high resolution (1024x600 pixels). This allows to easily share files, analyze results and more with tablet-style display and easy scroll and zoom functions. The screen is very resistant since it is covered with Amorplate glass.

- User experience and connectivity

The device incorporates two speakers, front-facing push buttons and 3D graphics accelerator to provide a real experience of use. Regarding the connectivity with the optical unit it has an UART access 3.3V TTL for serial port communication. To export files and manage with a computer or server the system incorporates Ethernet connection (10/100, RJ45) and a USB 2.0 Port.

- Low Power consumption

MYCORRAY reader power consumption is between 210-460mA@5V, depending on activity and processor speed.

Software

MYCORRAY device includes a whole software development that can be split in three main groups.

- MYCORRAY application

A user friendly application that guarantees an easy operation by non-specialised technicians has been designed, developed and implemented in the computational unit. Some of the main rules and elements of Material Design (by Google) have been considered in the design of the user interface.

Figure 2. Main Menu screen

MYCORRAY application has been developed including the steps/blocks of the detection process such as: take picture, validate cartridge, start spot detection process and save results.

Figure 3. Capture image, cartridge validation and image process

Several result screens are included to make easier to understand the results of the analysis by the end users. The application can generate an Excel File containing the fungi DNA list that have been detected in the analysed sample.

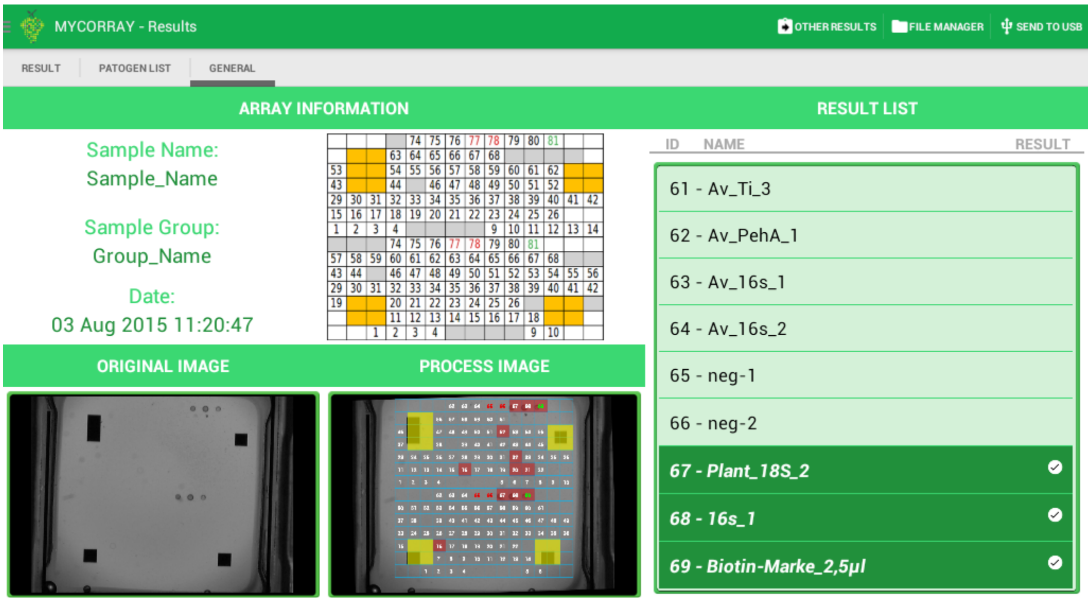


Figure 4. General screen of results

The application also have a special menu where the user can read basic information about MYCORRAY project, main objectives, competitive advantages of the system and basic information about the technology used, as well as information about the consortium partners.

- Detection algorithms based on image signal processing

MYCORRAY application incorporates a Java API developed to process and to interpret automatically the image obtained. For this purpose OpenCV has been used, an open source and widely extended library for computer vision.

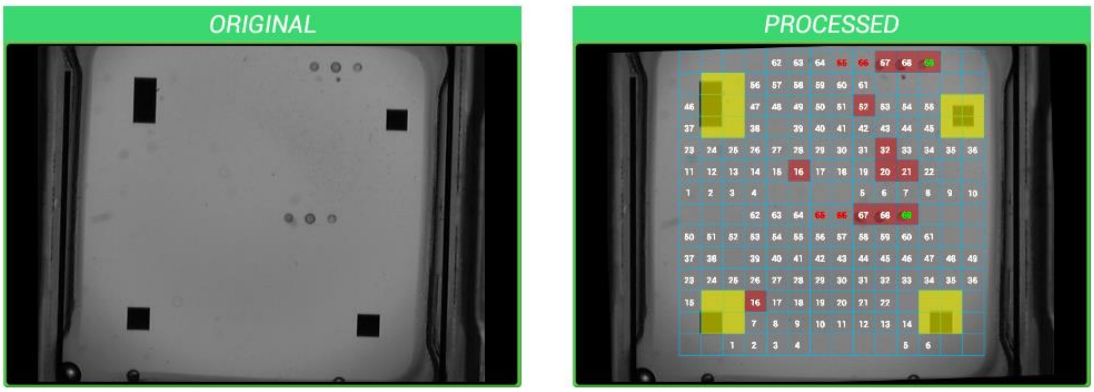


Figure 5. Original and Processed array capture analyzed by MYCORRAY application

The processing algorithm to detect the spots follows two steps: first of all, to validate the cartridge there is a validation block which detects the reference squares filtering and analysing the image, rotate to correct the position of the array and flip it if necessary and, secondly, the system detects the spots related to the pathogens using special algorithms. Due to the backlight changing depending on the array tube, the application includes a smart algorithm which change thresholds and some parameters of the detection process in order to always achieve the best result.

MYCORRAY analysis is qualitative, not quantitative. The detection threshold is for concentrations above 20 µg/ml.

- Device customization and external apps

MYCORRAY reader has been fully customized as a commercial Android device, from the kernel to the operative system personalization layer.

A new launcher for Android has been developed. Launcher is the name given to the part of the Android user interface that lets users customize the home screen giving a better appearance to the device and making the main functionalities more accessible.

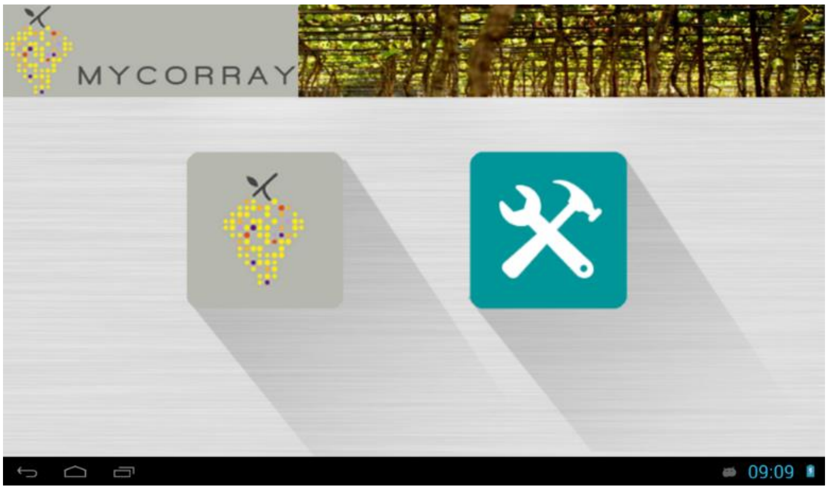


Figure 6. Launcher. Home screen of MYCORRAY device.

Also, MYCORRAY includes an external application to make easier and safer the import/export of MYCORRAY files generated during the analysis. This is a File Manager which allows the user to access only to the files generated by MYCORRAY application hiding the system files. It allows to export, copy, edit and move files and folders to a USB drive and vice versa. This application is accessible from the MYCORRAY application and from the launcher.

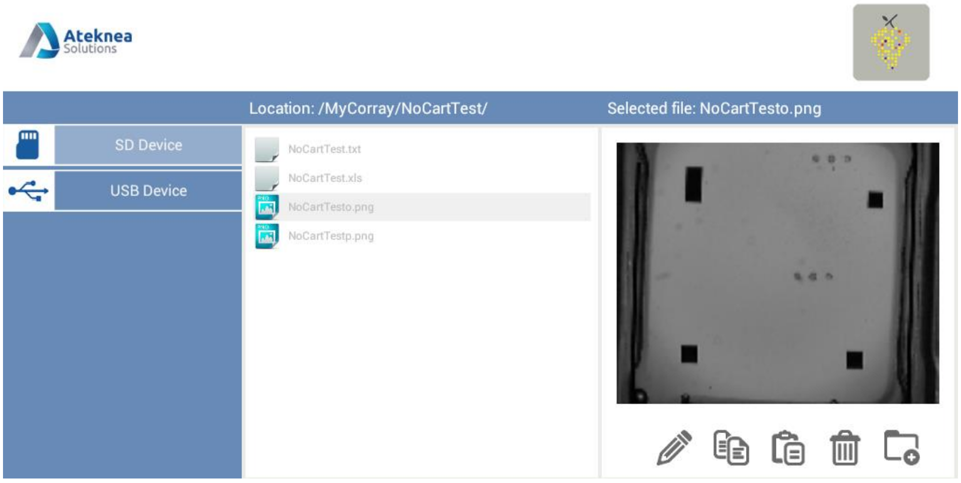


Figure 7. File manager of MYCORRAY device.

Main technological result: the MYCORRAY cartridge

The MYCORRAY system consists of a disposable array tube containing a DNA microarray chip and a cost-effective standalone array reader suitable for use by non-specialists. The Microarray chip has been designed to detect 12 of the most important fungal species involved in grapevine trunk disease; these include *Neofusicoccum parvum, N. luteum, Diplodia seriata, Botryosphaeria dothidea, Lasiodiplodia theobromae, Phaeomoniella chlamydospora, Phaeoacremonium aleophilum, Fomitiporia mediterranea, Eutypa lata, Ilyonectria liriodendra, I. macrodidyma* and *Diaporthe neoviticola*. In addition, the array can be used to detect the presence of the bacterium *Agrobacterium vitis* and distinguish between strains harbouring the Ti plasmid. The final iteration of the array also includes control probes for generic detection of plant, bacterial DNA to be used as internal controls. The array is highly specific allowing sensitive and accurate detection of grapevine pathogens and can reduce the time required to obtain results.

Methods

Specific primers and probes were designed for each of the target organisms. Primers and probes were designed for 12 fungal pathogens and the bacterial pathogen *Agrobacterium vitis*. Multiple genes were selected for each target on the basis of the availability of sequence data and the suitability of each gene to discriminate the target from non-target species and also to inclusively detect sequence variants. The representation of each pathogen by multiple genes allowed us to determine which genes were the most promising targets for inclusion on the final operational version of the array, and was particularly important for pathogens for which the taxonomy is relatively unclear.

The array was developed in an iterative process involving several rounds of design and testing of both probes and primers, this included the following steps: alignment of target and non-target sequences for each species/gene combination; probe design for specificity and inclusive detection of variants; primer design; screening of primers; testing of selected PCR products on the array to assess probe performance; selection of probes; selection of best performing primer pairs. The arrays containing the selected probes were manufactured by Alere in an Array Tube format.

The best performing primer pairs were selected for use in the PCR using biotin-labeled nucleotides, PCR products were tested on the array. These results were used to identify the best performing probes in terms of (i) signal strength with target PCR product(s); (ii) absence of cross reactivity with non-target PCR products; (iii) consideration of the level of redundancy for each target. Concurrently, preferred primer combinations were identified on the basis of (i) target signal strength and (ii) the absence of non-specific background or cross reactivity with non-target probes.

Once the primers and probes had been selected the multiplex PCR was optimised, this involved the addition of bovine serum albumin (BSA) and dimethyl sulfoxide (DMSO) to reduce the production of non-specific DNA products.

Results

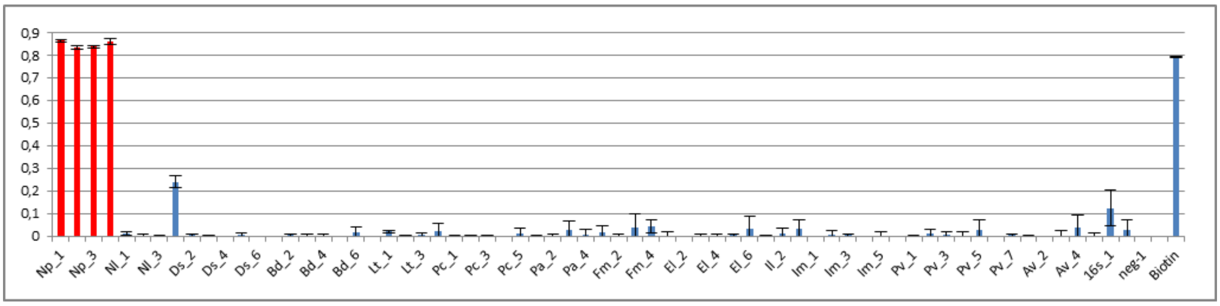
A total of 46 alignments were produced during the development of the array, from these 120 target-specific probes were designed, representing multiple genes for each species. The total number included a degree of redundancy such that multiple probes were designed for many of the target sequences. Primers were designed for each species/gene combination; multiple primer pairs were designed for each target which in some cases covered different sub-sets of the probes for the relevant target. 135 primer combinations were screened by real-time PCR using a fluorescent dye (EvaGreen), and primer pairs were selected (46 pairs) for further testing on the basis of (i) amplification of target DNA; (ii) lack of amplification in the no-template control; and (iii) presence of a discrete melting peak indicating the efficient production of a single, specific amplification product.

The multiplex PCR contains a total of 34 primer pairs; in order to maintain sensitivity for detection of all targets, these were combined into four smaller multiplex combinations (comprising 14, 7, 7 and 6 primer pairs, respectively). This approach has the advantage that new primer pairs can more easily be added at a later stage, increasing flexibility of the system. The final iteration has a total of 69 probes, 64 are for the target pathogens and the remaining 5 are control probes.

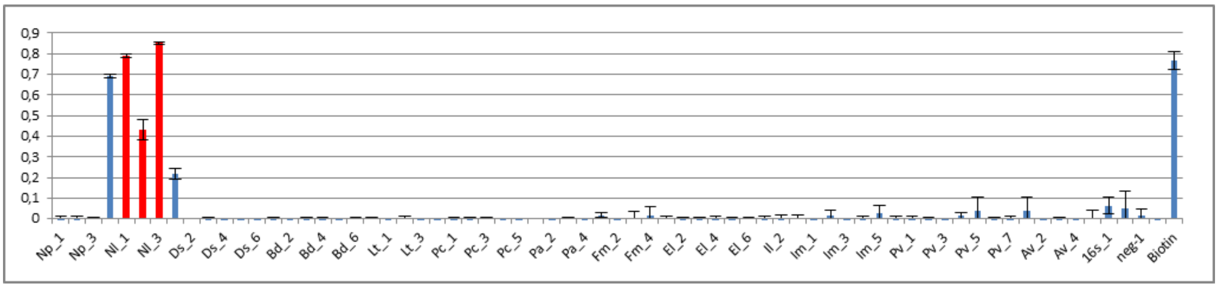
The results (figure 1 A-M) show that in each case the target species were detected, but cross reaction was seen with one probe for *Diplodia seriata* (DS_1).

Figure 8: histograms show the mean (of two replicates) spot intensity for all spots on the array when testing (A) *Neofusicoccum parvum,* (B) *Neofusicoccum luteum*, (C) *Diplodia seriata,* (D) *Botryosphaeria dothidea,* (E) *Lasiodiplodia theobromae,* (F) *Phaeomoniella chlamydospora,* (G) *Phaeoacremonium aleophilum,* (H) *Fomitiporia mediterranea,* (I) *Eutypa lata,* (J) *Ilyonectria liriodendra,* (K) *Ilyonectria macrodidyma,* (L) *Phomopsis viticola,* (M) *Agrobacterium vitis*. Target-specific signals are highlighted (in red) for each target. The blue lines on the right of the histograms are the control lines for biotin and bacterial DNA,

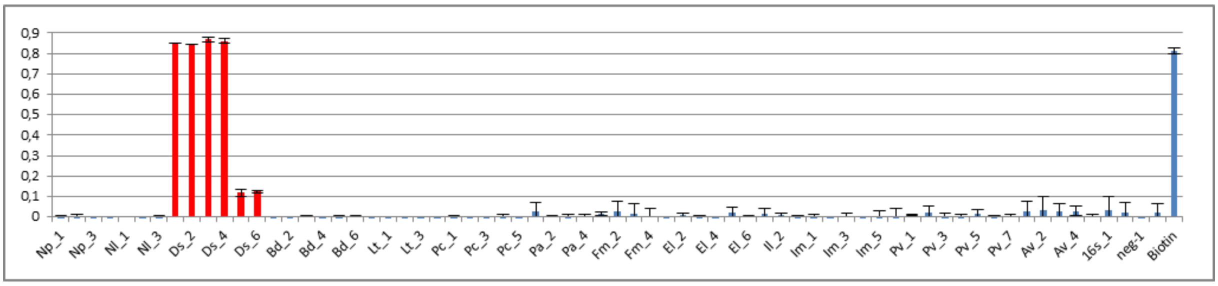
A. *Neofusicoccum parvum*



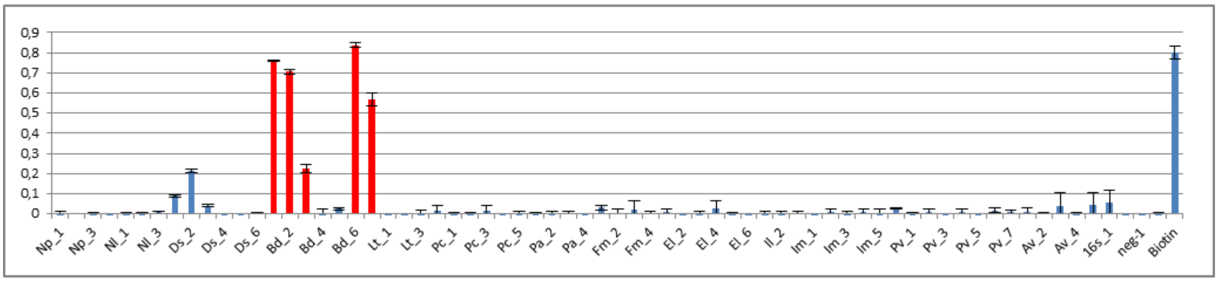
B. *Neofusicoccum luteum*



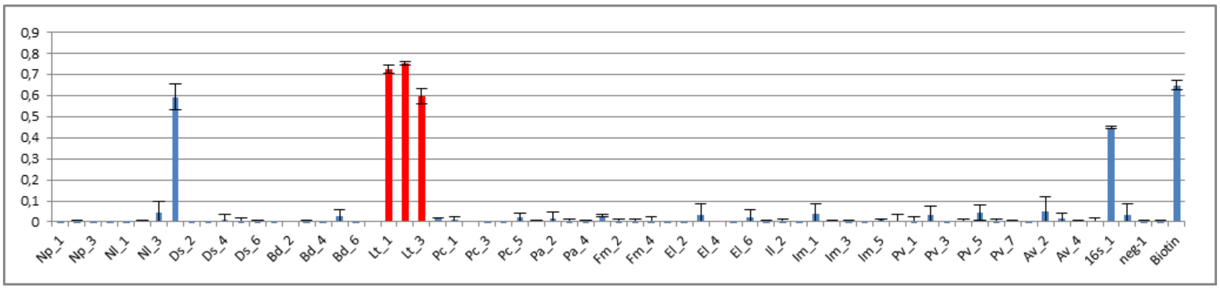
C. *Diplodia seriata*



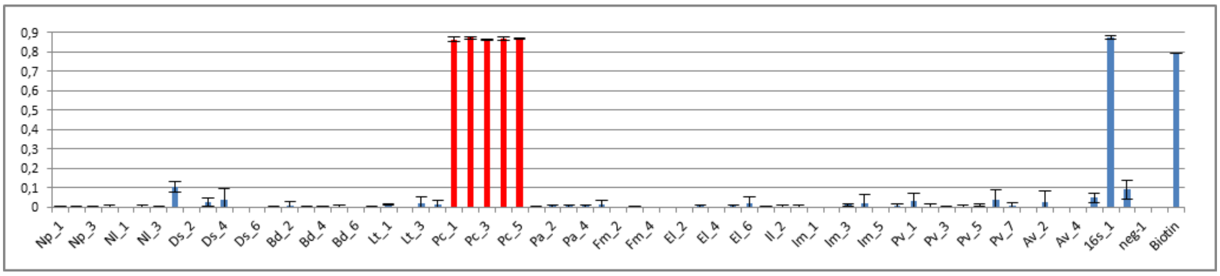
D. *Botryosphaeria dothidea*



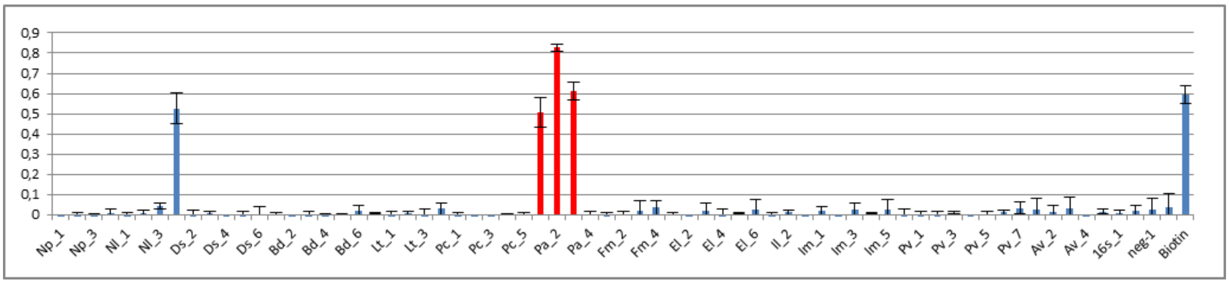
E. *Lasiodiplodia theobromae*



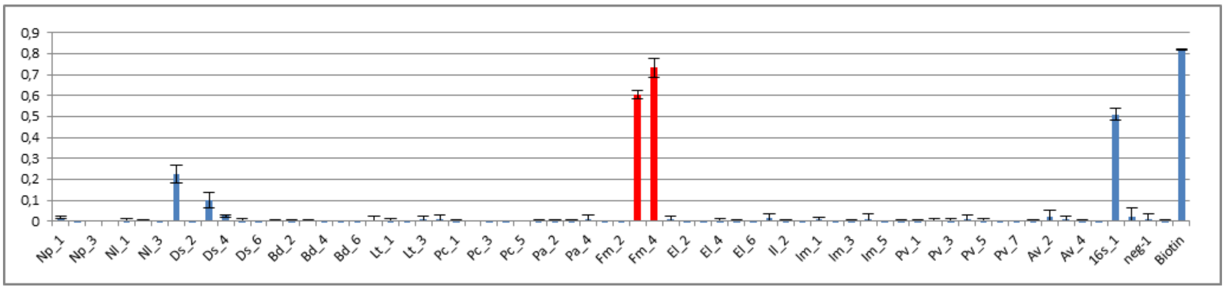
F. *Phaeomoniella chlamydospora*



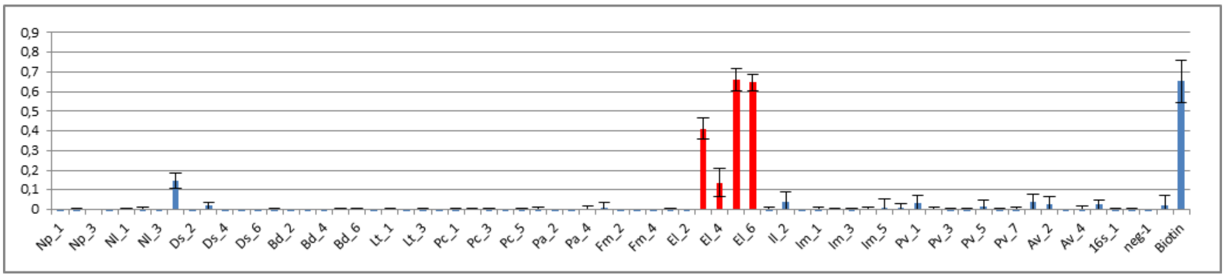
G. *Phaeoacremonium aleophilum*



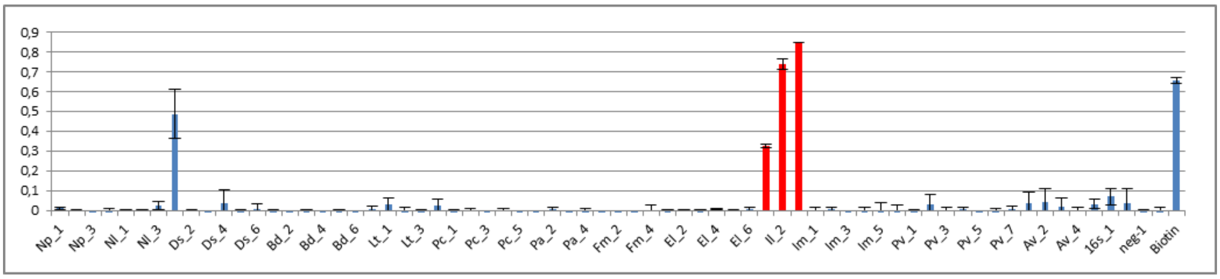
H. *Fomitiporia mediterranea*



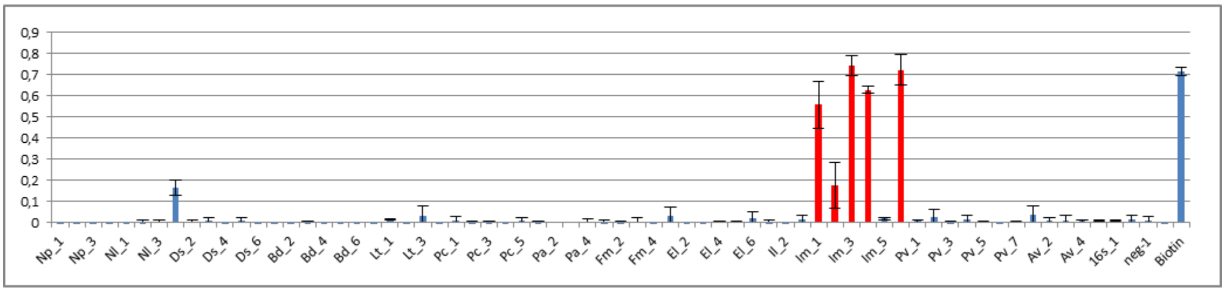
I. *Eutypa lata*



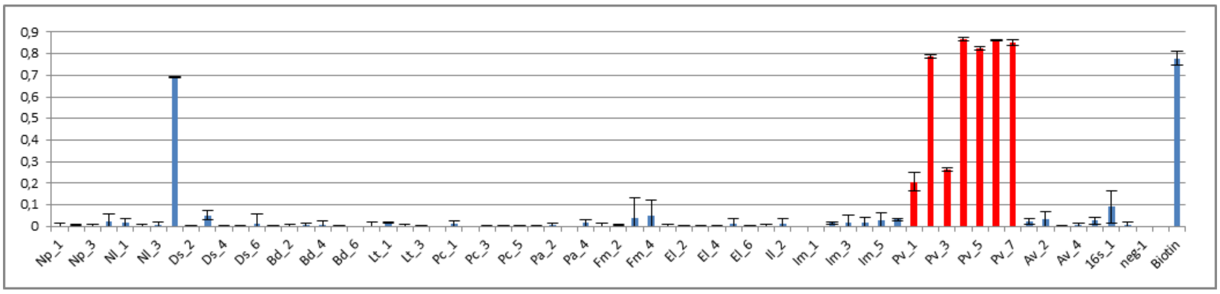
J. *Ilyonectria liriodendra*



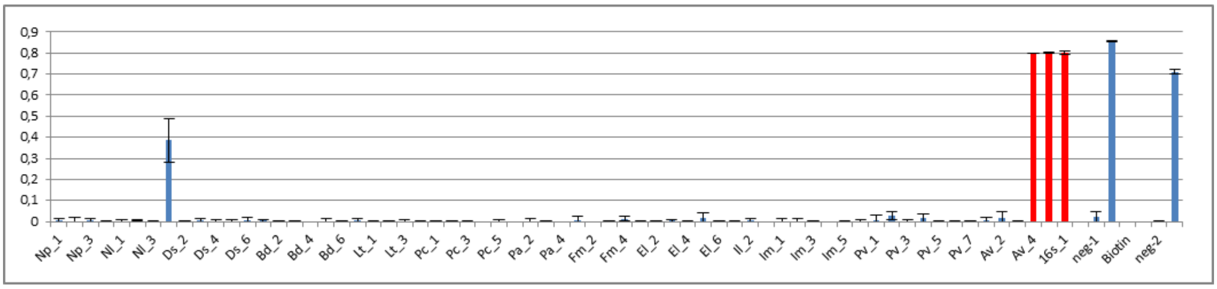
K. *Ilyonectria macrodidyma*



L. *Phomopsis viticola*



M. *Agrobacterium vitis*



Scientific result: sampling protocols

A protocol for sampling standing vines and nursery material was developed together with the selection of the best DNA extraction method from grapevine woody tissues.

DNA extraction method

DNA extraction from wood encounters several problem related to chemical and physical features of the material. For this reason the powdering of the sampled woody tissues prior DNA extraction was introduced in the protocols used in the project. Particularly, the problems are related to the thick cell walls of wood that naturally contain numerous chemical compounds, such as phenolic compounds of the lignin biosynthesis pathway, strong inhibitors for DNA extraction. Moreover, plant polysaccharides can bind the DNA and prevent the extraction of a usable DNA for PCR reactions. For this reasons, different DNA extraction methods were developed for eliminating contaminants from the purified DNA.

The selection of the best DNA extraction method was carried out comparing 2 CTAB based protocols (CTAB 2% and CTAB 3%) and 5 protocols based on commercial kits. The evaluation of the quality of DNA extracted was tested by verifying its amplificability both by Real Time and end-point PCR. Moreover the measurements of total DNA concentration (A260) in the samples was done by means of spectrophotometric readings. In the selection of the different extraction methods also the cost for each reaction and the time required for the analysis were considered.

It was found that DNA reading at A260 in the samples was prevented probably by the presence of compounds that can interfere with the measurement. It is well known that phenols contribute to the total signal in an A260 measurement being not discernible from double strand DNA. Nevertheless determination of total and fungal DNA amplifiability by real-time and end-point PCR indicated consistent differences in the quality of DNA extracted with the different methods.

Particularly, the DNAs extracted with MACHEREY NAGEL NucleoSpin Plant II kit amplified well by real time PCR, showing a low average Ct value, and were those that better amplified with end-point PCR. Since this commercial kit is one of the less expensive and time consuming among those tested and it provided the most reproducible results, it was chosen as the most suitable for project purposes.

Sampling of standing vines

Until now indexing the agents of grapevine decline was mainly performed by uprooting or cutting at the base the vine trunk and extracting DNA or performing the standard isolation methods from the internal woody tissues, thus causing an economical damage to the vineyard owners. For the purpose of MYCORRAY project, it was proposed a non-lethal processing method for the standing vines. Woody tissues were collected by means of drilling taking into account that the different types of wounds that can be found on the trunk represent the primary site of infection or recurrent sites of re-infection for most of the fungi of interest. The samples collected were pulverized by mixer mill prior to DNA extraction. After the sample collection the holes that remained in the vines were protected to prevent new fungal infections.

Sampling of nursery material

Plant material from nursery (cuttings, rootstock etc.) can be processed by opening the sample and selecting the tissues for collecting the wood. Symptomatic tissues showing necrosis or discoloration in this case can be chosen and sampled. The processing method consists of an initial cleaning and disinfection of the nursery material. Then the bark was removed and from the internal symptomatic or asymptomatic tissues the wood was collected. The wood was pulverized by a mixer mill as in the case of standing vines samples, and the DNA extracted.

Sampling strategy

To select the best sampling strategy single and pooled samples were collected both for standing vines and nursery material. Comparing the results of the detection analyses for some of the fungi included in the project, the pooled sample showed to be the most suitable, allowing to reduce the number of samples collected and analysis performed, reducing the costs and representing a great step forward for the end-users.

Scientific result: MYCORRAY laboratory validation

The MYCORRAY system has been validated on a laboratory scale, including the assessment of specificity (inclusivity and exclusivity) and the limit of detection for each pathogen. The array contains probes for the detection of 12 fungal pathogens and the bacterial pathogen *Agrobacterium vitis*, with probes for multiple genes per organism. The array also includes control probes for generic detection of plant, and bacterial DNA to be used as internal controls. The array has also been validated using grapevine samples from the field, these samples have been tested using the array, real time PCR and nested PCR.

The MYCORRAY system was initially validated using laboratory cultures of well characterised isolates for each of the grapevine trunk pathogens. Target pathogens (30 in total) were tested on the array (see Table 1). Where possible multiple isolates from different geographical regions were selected to challenge the inclusivity of array. All target pathogens were detected on the array.

Table 1: Target pathogens tested on the array

Target pathogen	**Abbreviation**	**Country of origin**	**Number of isolates tested**	**Number of isolates detected**
Neofusicoccum parvum	Np	Venezuela, New Zealand and UK	3	3
Neofusicoccum luteum	Nl	New Zealand and USA	2	2
Diplodia seriata	Ds	Italy and UK	4	4
Botryosphaeria dothidea	Bd	Italy and Japan	3	3
Lasiodiplodia theobromae	Lt	Italy	1	1
Phaeomoniella chlamydospora	Pc	Italy	2	2
Phaeoacremonium aleophilum	Pa	Italy	2	2
Fomitiporia mediterranea	Fm	Italy and unknown	3	3
Eutypa lata	El	France and unknown	2	2
Ilyonectria liriodendri	Il	UK and unknown	3	3
Ilyonectria macrodidyma	Im	Unknown	2	2
Diaporthe neoviticola (Phomopsis viticola)	Pv	UK and Italy	2	2
Agrobacterium vitis	Av	Unknown	1	1

Non target organisms were tested on the array to ensure that they would not cross react with the probes. Closely related non-pathogens were selected based on their genetic similarity to the target pathogens and their likelihood of infecting grapevines; 25 non target organisms were tested on the array. In addition a further 10 organisms that had been isolated from grapevines were tested.

Non target organisms were initially tested as neat DNA extracts from the cultures to challenge the array with unnaturally high concentrations of these organisms. For the organisms that cross reacted under these conditions testing was then repeated at a DNA concentration more representative of a natural infection (100pg/µl). *Botryosphaeria cortices* and *Botryosphaeria berengeriane* were detected with probes on the array at 100pg/ul. Only 1 of the genes for *Botryosphaeria dothidea* was detected, probes for the second gene were not detected. If results for only the first gene are displayed this would indicate that further testing would be required. *Neonectria radicicola* was also detected on the array; this organism is very closely related to *Ilyonectria liriodendra* and is also involved in black foot disease of grapevines. *Eutypa ameriacae* was detected on the array. Again this is also a grapevine pathogen of interest to the industry and it was unable to distinguish between these two closely related species using the array.

To establish the sensitivity of the array target pathogens were cultured and DNA was extracted, the concentration of the DNA was established using the Quant-it assay (Life technologies). All DNA was diluted to 1000pg/ul and then a 10-fold serial dilution produced and tested with the array to establish the limit of detection for each of the target organisms. The sensitivity of the array varied from target-to-target and gene-to-gene, this is due to differences in efficiency of the PCR and binding for the different probes as well as the copy number of the various genes. The limit of detection for the majority of the targets is 10-100pg/µl (Table 2). Probes for *Phaeomoniella chlamydospora* are the most sensitive detecting DNA at 100fg/µl and 1pg/µl.

Table 4. Limit of detection for each gene for each target pathogen.

Target organism	Gene	Limit of Detection (pg/µl)
Neofusicoccum parvum	1	10
	2	100
	3	100
Neofusicoccum luteum	1	10
	2	100
Diplodia seriata	1	100
	2	100
	3	10
Botryosphaeria dothidea	1	1
	2	100
Lasiodiplodia theobromae	1	100
	2	100
Phaeomoniella chlamydospora	1	1
	2	100
Phaeoacremonium aleophilum	1	100
	2	10
Fomitiporia mediterranea	1	>1000
	2	10
Eutypa lata	1	100
	2	100
Ilyonectria liriodendra	1	1000
	2	1

Ilyonectria macrodidyma	1	100
	2	10
Phomopsis viticola	1	100
	2	100
	3	1
Agrobacterium vitis	1	100
	2	10
	3	100

When compared to the sensitivity of the real-time PCR (TaqMan) assays the array is generally 10-100 times less sensitive. This is to be expected; real-time PCR is generally 10-fold more sensitive than conventional PCR and multiplexing large numbers of primers into a PCR reaction also reduces the sensitivity of conventional PCR due to competition between the different primers. For the purposes of the MYCORRAY tool all target organisms can be detected at a level of 100pg/µl and in most targets at lower levels.

A panel of naturally infected (real) grape vine samples were tested in parallel using the new MYCORRAY system, the Clondiag array scanner, the conventional laboratory standard (nested PCR) as well as with the real-time PCR assays developed within this project. Field samples (88) were obtained from four countries; Italy (29 samples), Spain (28 samples), UK (23 samples) and France (8 samples).

On the whole the results from the four methods support each other, although there is no method for detection of *Fomitiporia mediterranea* other than the array so it so not possible to confirm the array results for this organism**.** Nested PCR assays were available for *Botryosphaeria* species, *Neofusicoccum parvum*, *Phaeomoniella chlamydospora*, *Eutypa lata*, and *Ilyonectria* species. Where there are discrepancies in the results this is mainly due to the difference in sensitivity in the assays. Real time PCR is 10-100 times more sensitive than the array and the nested PCR equal in sensitivity or 10 times more sensitive than the array depending on the target. The nested PCR targets the ITS gene and is therefore not as specific as the array or real time PCR. It was not possible to perform a side by side method comparison for true positives or true negatives as the samples were field samples with unknown infection levels. There is no gold standard method that has been reliably validated for all of the target organisms; it is therefore not possible to determine which of the methods represents the true results.

The MYCORRAY system can detect 13 pathogens simultaneously using a cost effective, standalone user friendly scanner, with the results displayed in a format that is easy to interpret. The MYCORRAY system is specific and fit for purpose; it can detect pathogens in grape vine material collected from across Europe. The MYCORRAY workflow is simpler than performing numerous separate real time PCR assays or nested PCRs for a single sample to determine the same information. Furthermore the approach of nested PCR requires samples to be subjected to DNA sequencing to confirm the exact organism present; this is both time consuming and expensive. There are not real time PCR and nested PCR assays for all 13 of the target pathogens, giving the MYCORRAY system an advantage in its ability to detect a wide range of pathogens in a single test.

The array is highly specific allowing sensitive and accurate detection of grapevine pathogens and can reduce the time required to obtain results. The MYCORRAY system has been validated in terms of sensitivity, specificity and using samples gathered from a range of vines from different countries and vine ages.

Potential impacts, main dissemination activities and foreseen strategy for the exploitation of results

The MYCORRAY system developed in this project compares very well with the state-of-the-art in terms of sensitivity and specificity (as it is also based on DNA amplification), but progresses beyond in terms of simultaneous detection, test and platform price, time-to-result, ease of use and interpretation, and self-sufficiency limitations. Simultaneous detection of 13 pathogens in a single tests, total required time per test 6h, but only 2h hand-on time, price per platform is expected to be not more than 2,000€ and 50€ per test. The platform performs the analysis and help the technician to interpret the results. Moreover, it is a standalone device with touch screen interface to operate and display the results.
This project has also developed the required knowledge regarding sound sampling strategies and sample processing methods, as well as knowledge related with the correlation between actual pathogen populations and phytosanitary status of vineyards and nursery plantations to provide end-users with a tool that will allow them making economically sound and effective decisions and to monitor the effect of these decisions.

The manufacture and commercialization of the MYCORRAY system, its application in vineyards and nurseries as well as its use by diagnostic test provider laboratories has direct economic and strategic competitive impact for all SME partners.

The MYCORRAY project has widespread social impact:

- **Promotion of employment**: In Italy, France and Spain, vineyard cultivation - and grape harvesting in particular - is an important source of employment. MYCORRAY will be cost-effective and user friendly and will allow to increase the knowledge base of winegrowers in these regions while preserving and expanding their business, thus supporting employment.
- **Environmental protection and sustainable production:** The developed project will facilitate the implementation of agro-environment schemes that address environmental problems of the sector by encouraging improved and sustainable procedures.
- **National and transnational authorities** will benefit from MYCORRAY when monitoring the evolution and occurrence of the disease, with significant associated public costs.
- **Health and safety** to consumers and EU citizens in general will benefit from reduced need to use pesticides, which will have a significant impact on the health of EU citizens.
- **Rural development:** Rural areas, representing more than 90% of EU territory and containing more than half of the EU population, face challenges related to growth, jobs and sustainability. MYCORRAY will facilitate the implementation of recent European rural development programmes.
- **Knowledge and quality of work and life**: This project will also help to increase the quality of work and producers satisfaction, providing them the knowledge to better understand the principles of the technology and to benefit from it by improving the management of their vineyards.

The MYCORRAY website (http://www.mycorray.eu) is the main digital dissemination channel and has been updated whenever a new event was done. Information about the project can also be found in respective partners' websites. Regarding Social Media, the hashtag #mycorray has been used to announce on twitter different meetings and dissemination activities, which have been also published on partners' Facebook accounts.

All Consortium Members did a wide variety of dissemination actions during all the project's lifetime. Almost 50 different activities are accounted, between workshops, exhibitions, conferences, presentations, courses, fairs and interviews. During each of them, the partners that assisted used one of the MYCORRAY prototypes to show the technology and how the tests are done.

Following the completion of the project, the MYCORRAY system will be further developed into a marketable product by a group of Consortium Members who will bring it to the market in the shortest possible time.

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