Training course material

A.2 – Training sessions and design of materials for demonstration trials

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Deliverable Name: Training course material

Action A.2: Training sessions and design of materials for demonstration trials

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1. Summary

Preparatory actions will provide information on the demo areas and design the respective trials. A.2 will design the materials and carry out training sessions for the demonstration trials. This action will prepare the relevant staff for the demonstration trials that will take place during 36 months of the project. Since many of the relevant farm staff of the demonstration sites are not familiar with the techniques and technologies that will be applied, these training sessions will be important to make sure the experimental methodology is accurate, and the results can be properly implemented and evaluated.

2. Introduction

Action A.2 is aimed at a solid replicability and transferability of the project to more farms across the European Union (EU). In order to replicate the best-practices demonstrated in the project, the farmers will need to be trained on the matter. They will benefit professionally and personally from training related to vector accounting and control, efficient water use, the positive benefits of biological control and the use of auxiliary flora and fauna. Apart from being technical, the training will also show the economic and environmental gains of the action. The consortium believes that by offering opportunities for upskilling and economic growth, more farmers will be inclined to participate in the replications, sustaining jobs in agriculture. Furthermore, the ultimate result of the best-practices is to generate a balanced ecosystem within the agricultural field, complete with healthy soil and plants. These healthy, balanced fields will be stronger and more resilient in the face of climate change and attacks from pests and pathogens, with a greater possibility of high yield in the future, keeping their farms competitive on the market and safeguarding their jobs for years to come.

3. Chapters + Results

The course includes the following chapters:

- Chapter I: Introduction to *Xylella fastidiosa*;

- Chapter II: Best practices to increase resilience; and

- Chapter III: Monitoring Activities.

Chapter I. Introduction to *Xylella fastidiosa*

1. Overview and chronology about the plant pathogen *Xylella fastidiosa*, a new threat in Europe

The plant pathogen *X. fastidiosa* (Wells *et al.*) (hereinafter, *Xf*) (Xanthomonadaceae, Gammaproteobacteria) is a xylem-limited Gram-negative bacterium that causes disease in important crops and ornamental plants. Its specific name denotes two characteristics of this bacterium: that it lives in the xylem of host plants (*Xylella*) and that it has a very slow growth in microbiological culture media (*fastidiosa*).

The first known syndrome caused by *Xf* was described in 1887 on grapevine in Los Angeles (California), being this pathology named as Pierce disease (PD) in 1930. *Xf* was isolated in 1978; in 1987 was properly described and classified and; in the year 2000 it became the first phytopathogenic bacterium from which the whole genome was sequenced [*Xf* clone 9a5c, (citrus strain)].

The bacterium was detected and eradicated in 2012 in France; in a greenhouse of coffee plants (*Coffea arabica* and *C. canephora*) which came from Ecuador and Mexico. The first outbreak of *Xf* (*Xf* subspecies *multiplex*) was reported in Corsica, France in 2015 on plants of the ornamental species *Polygala myrtifolia*; and at the end of that same year, it was detected in Nice, Alpes-Maritimes and PACA Region.

The identification of *Xf* was reported for the first time in Europe (*Xf* subsp. *pauca*) at the end of 2013, when detected on aged olive trees, almonds and oleanders in the Apulia region of Southern Italy. However, years before, some strains of the pathogen [*Xf* subspecies *multiplex* and *fastidiosa* (*Xff*)] had already been discovered on symptomatic olive trees in California urban areas. By the end of 2013, the presence of *Xf* subsp. *pauca* was confirmed in olive orchards from Argentina. In 2016, in a greenhouse of a small nursery in Saxony, Germany, *Xff* was detected in oleander and rosemary plants; and few months later in *Streptocarpus* sp. and *Erysimum* hybrids.

At the end of 2016, in Mallorca (Spain), *Xf* was detected in cherry trees and plants of *P. myrtifolia*. The first detection of *Xf* in olive trees in Spain (Villarejo de Salvanés, Madrid) was described on April 2018 in a symptomatic Picual cultivar olive tree. The

presence of the pathogen was confirmed for the first time in the country on June 2017 in an almond orchard in Alicante province, and – almost one month later – in almond trees about 1 km away from the first detection. Asymptomatic polygala (myrtle-leaf milk-wort) plants were reported positive to *Xf* in an insect-proof net greenhouse of a nursery in Almería province (Andalusia, Spain) on April 2018. Around one year later (May and July 2018), new positive results for *Xf* in almond trees and in other plants typical of the Mediterranean ecosystem were detected, both within the demarcated area of Alicante in mainland Spain. On November 2018, new positives for *Xf* [*multiplex* subspecies and sequence type (ST) 6 strain] were reported in the currently declared demarcated area of Alicante, including the first European detection of the bacterium in an apricot tree.

As early as December 2018, *Xf* subsp. *multiplex* was found for the first time in Tuscany, being different from the Apulian subspecies isolated in Italy so far. The subspecies *multiplex* detected in the Tuscan territory – also present in France and Spain –, was positive in several trees and shrubs but not in olive trees. In January 2019, *Xf* subsp. *multiplex* was detected in Portugal on lavender plants from a garden.

2. Biology and ecology of *Xylella fastidiosa*, how is the pathogen spread

Xf is a bacterium with a dual lifecycle, colonizing two habitats: the xylem of the host plant that infects – where it may or may not cause disease –, and the anterior intestine of the gross sap sucking insects which are vectors that transmit the bacterium. *Xf* is transmitted – from an infected plant to a healthy one –, and disseminated persistently – among fields at short and medium distances – by different species of sucking insects of the xylem of the hosts is affects. In the 1940s, the transmission of PD by vectors was demonstrated. Another way by which the pathogen can be introduced in a region distant from the original infection site is the use of planting material or that used for grafting from infected plants. Moreover, the insect vector may also preserve the bacterium several months after acquiring it, thus allowing *Xf* to infect plants in a new area. The virulence of *Xf* is related with some functional traits such as movement and xylem vessels colonization, which is essential for bacterium subsistence.

The lifecycle of *Xf* includes several steps which are crucial in the bacterium transmission. It begins when an adult insect vector punctures an infected plant colonized by the bacterium and then sucks the *Xf* cells (ingestion). This step could be favored by the higher concentration of some structural polysaccharides (pectin and glucan) as a result of the bacterial degradation activity of the plant xylem walls. Once an enough number of insect-colonizing bacterium is inoculated by the vector, and then it punctures another target plant, the bacterium is detached from the insect foregut and dispersed by the tracheal elements or by the cells of the xylem propagating the infection.

In susceptible host plants – following the proliferation of the local bacterial concentration from the site of infection – the bacterial cells of *Xf* colonize the xylem, with the consequent formation of colonies that increase their size on the inner xylem walls. Biofilm formation by the first bacterial colonies (adhesion step), which contain adhesins and complex exopolysaccharides, has been shown to be an important factor for plant colonization and infectivity of insect vectors.

3. Subspecies and genetic groups of *Xylella fastidiosa*

Genotyping methods based on DNA sequences to compare different isolates of *Xf* causing disease in a wide range of host plants evidenced that *Xf* was not represented by a homogeneous group of bacteria. At first, four major genetic groups were described and currently classified as subspecies: *fastidiosa* (isolates of grapevine, almond and alfalfa among other hosts); *pauca* (isolates of coffee, orange and olive trees among others); *multiplex* (several hosts including *Prunus* spp., *Quercus* spp., *Ulmus* spp., *Rubus* spp. and *Morus* spp.); and *sandyi* (oleander). There are strains of *Xf* that could not be assigned to any of the four subspecies. A fifth subspecies: *Xf* subsp. *tashke* was identified from isolates of *Chitalpa tashkentensis*, an ornamental species. More recently, a sixth subspecies, *Xf* subsp. *morus* was proposed for isolates that colonize mulberry trees (*Morus* spp.) in United States (US); and isolates from a shrub (*Nandina domestica*).

At present, the most commonly used way to assign *Xf* isolates – at the level of subspecies and sequence type (ST) or subgroup within a subspecies – is based on the amplification and sequencing of seven house-keeping genes or multilocus analysis (multilocus sequence typing, MLST). The isolation of a new different species of the genus (*X. taiwanensis*) on Asiatic pears (*Pyrus pyrifolia* and *P. serotina*) is suggested by the latest phylogenetic studies in 2016, and that further adds to the genetic reservoir of the pathogen.

Xf subspecies cause important diseases in crops different from olive trees. Among them the almond leaf scorch disease (ALSD), caused by several strains of *Xf* subspecies *multiplex* and *fastidiosa*; the citrus variegated chlorosis (CVC), caused by the subspecies *pauca*; the phony peach disease (PPD), caused by the subspecies *multiplex* and *fastidiosa*; and, importantly, the Pierce's disease of grapevines caused by *Xff*. Similar to olive trees, almond cultivars are differentially resistant to ALSD.

The pathogenicity and virulence of the *Xf* subspecies depend of their genetic structure and biology, being different their potential to induce diseases in certain host plants. In the case of the olive trees, the bacterium pathogenicity is determined at the level of the subspecies (e.g. Italian, Argentinian and Brazilian strains of *Xf* subsp. *pauca*). Moreover, the severity and the time of symptoms appearance caused by the *Xf* infection differ with the olive cultivar (see Section 6 for more information).

Xf subsp. *pauca* isolated in Apulia orchards (CoDiRo or Salento-1 strain) seems to have been introduced with ornamental coffee plants from Costa Rica (ST53 strain). The strain ST53 of *Xf* subsp. *pauca* isolated in Apulian olive trees was found to be the same as that identified in France in 2015 on infected polygala plants from a botanical garden with old olive trees. The strain of *Xf* subsp. *pauca* characterized in olive trees of Argentina was previously undescribed and different from the Italian one, as in the case of Brazilian strain ST16.

Xf was recently detected also in Iran on almond trees and grapevines with PD symptoms being unclear whether the isolated subspecies is indeed *fastidiosa*. The origin of *Xf* subsp. *multiplex* detected in Tuscany is not known, but among the most reliable

hypotheses there is the introduction of infected plants into the Tuscan region or the transport of insects carrying the bacterium, coming from areas outside the Italian territory.

4. Susceptible host plants to *Xylella fastidiosa* in the European Union

According to the evaluation of the European Food Safety Authority (EFSA) in September 2018, the list of *Xf* host plants is extremely wide, including 563 plant species identified (see Table 1). Its database is increasing, suggesting that *Xf* could affect other susceptible host plants (crop, ornamental, forestry or wild species) that are found in the new European outbreaks and/or not currently described.

Table 1. Host plants found to be susceptible to different subspecies of *Xylella fastidiosa* in the Union territory, according to the latest update of the European Commission database of 19 September, 2018

Subspecies of *Xf*	Susceptible host plant
Xf subsp. *fastidiosa*	*Cistus monspeliensis* L.
	Erysimun
	Juglans regia L.
	Prunus avium L.
	Streptocarpus
	Vitis vinifera L.
Xf subsp. *multiplex*	*Acacia dealbata* Link
	Acacia saligna (Labill.) Wendl
	Acer pseudoplatanus L.
	Anthyllis hermanniae L.
	Artemisia arborescens L.
	Asparagus acutifolius L.
	Calicotome villosa (Poiret) Link
	Cercis siliquastrum L.
	Cistus creticus L.
	Cistus monspeliensis L.
	Cistus salviifolius L.
	Convolvulus cneorum L.
	Coronilla glauca L.
	Coronilla valentina L.
	Cytisus scoparius (L.) Link
	Cytisus villosus Pourr.
	Euryops chrysanthemoides (DC.) B.Nord.
	Ficus carica L.
	Fraxinus angustifolia Vahl
	Genista x *spachiana* (syn. *Cytisus racemosus* Broom)

	Genista corsica (Loisel.) DC. *Genista ephedroides* DC. *Grevillea juniperina* R. Br. *Hebe* *Helichrysum italicum* (Roth) G. Don *Lavandula angustifolia* Mill. *Lavandula dentata* L. *Lavandula stoechas* L. *Lavandula* x *allardii* (syn. *Lavandula* x *heterophylla*) *Lavandula* x *intermedia* *Lonicera japonica* Thunb. *Medicago sativa* L. *Metrosideros excelsa* Sol. *ex* Gaertn. *Myrtus communis* L. *Olea europaea* L. *Pelargonium graveolens* L'Hér *Phagnalon saxatile* (L.) Cass. *Prunus avium* (L.) L. *Prunus cerasifera* Ehrh. *Prunus domestica* L. *Prunus cerasus* L. *Quercus suber* L. *Rhamnus alaternus* L. *Rosa canina* L. *Spartium junceum* L. *Westringia fruticose* (Willd.) Druce
Xf subsp. *pauca*	*Acacia saligna* (Labill.) Wendl *Asparagus acutifolius* L. *Catharanthus* *Chenopodium album* L. *Cistus creticus* L. *Dodonaea viscosa* Jacq. *Eremophila maculata* F. Muell. *Erigeron sumatrensis* Retz. *Erigeron bonariensis*L. *Euphorbia terracina* L. *Grevillea juniperina* L. *Heliotropium europaeum* L. *Laurus nobilis* L. *Lavandula angustifolia* Mill. *Lavandula stoechas* L. *Myrtus communis* L. *Myoporum insulare* R. Br. *Olea europaea* L. *Pelargonium* x *fragans* *Phillyrea latifolia* L.

	Prunus avium (L.) L. *Rhamnus alaternus* L. *Spartium junceum* L. *Vinca* *Westringia fruticosa* (Willd.) Druce *Westringia glabra* L.
Xf - irrespective of the subspecies	*Calicotome spinosa* (L.) Link *Cistus albidus* L. *Coffea* *Genista lucida* Cambess. *Helicrysum stoechas* (L.) Moench *Lavandula dentata* L. *Lavandula* x *chaytorae* *Nerium oleander* L. *Polygala myrtifolia* L. *Prunus dulcis* (Mill.) D.A. Webb *Rosmarinus officinalis* L. *Teucrium capitatum* L. *Veronica elliptica* L.

Source: European Commission (2018).

5. Insect vectors, the vectors carrying the *Xylella fastidiosa* phytobacterium

Xf is adapted to subsist into arthropods being most of these insect vectors. Thus, the pathogen transmission occurs by these sucking insects which behave as vectors carrying

Table 2. Vectors of *Xylella fastidiosa* in the American continent

Insect group	Most important species	Distribution	Role as vector	Role as vector: criteria
Sharpshooters (Cicadellidae, Cicadellinae): 38 species	*Bucephalogonia xanthophis* (Berg)	Neotropical: Argentina, Bolivia, Brazil, Paraguay	High in citrus	Common, abundant on ornamental plants, citrus and nursery stocks
	Dilobopterus costalimai Young	Neotropical: Brazil	High in citrus	Common, abundant on ornamental plants and citrus
	Graphocephala atropunctata (Signoret)	US and Central America	High in grapevine	Common in diverse ecosystems, on grapevine and ornamental plants

	Homalodisca vitripennis (Germar)	US (Southern States), Mexico (northern part), French Polynesis, Easter Island	High in grapevine	Common and abundant in diverse ecosystems, on grape, ornamental, citrus and nursery stock
Spittlebugs (Cercopoidea): six spp.	*Philaenus spumarius* L.	US including Hawaii, Mexico, Tahiti	Low	Not associated with disease epidemics
Cicadas (Cicadoidea): two spp.	*Diceroprocta apache* Davis *Dorisiana viridis* (Olivier)	Mexico, Arizona, Utah, Nevada, California	Doubtful	Missing information on transmission capacity

Source: EFSA (2015).

the bacterium and are able to achieve the xylem in the infected tissues of the plant and to suck the raw sap containing the bacteria. These species of xylem-feeding insects belong to the order Hemiptera, suborder Auchenorryncha, infraorder Cicadomorpha, and more specifically to the families Aphrophoridae, Cercopidae, Cicadidae and Cicadellidae.

Figure 1. Vectors of *Xyella fastidiosa* (A) *Philaenus spumarius*, Michaelson, J.; (B) *Cercopis vulnerata*, Farrell, S.; and (C) *Cicadella viridis*, Bantock, T. (Malumphy C., 2017).

The exotic vector *Homalodisca vitripennis* or glassy winged sharpshooter (Hemiptera: Cicadellidae) – very efficient in the transmission of *Xf* and difficult to control – was introduced from Mexico in California in the 1990s, causing a remarkable increase in the incidence of PD. In Apulia, *Xf* is transmitted by *Philaenus spumarius* (Hemiptera: Aphrophoridae) (Figure 1A), which has proven to be an extremely efficient and abundant vector.

Table 3. Potential vectors in Europe that could contribute to the spread of *Xylella fastidiosa*

Family	Species	Distribution
Aphrophoridae	*Aphrophora alni*, *A. major*, *A. pectoralis*, *A. salicina*, *Lepyronia coleoptrata*, *Neophilaenus campestris*, *N. exclamationis*, *N. lineatus*, *N. longiceps*, *Philaenus spumarius*, *P. italosignus*	France, Italy, Spain, United Kingdom (UK)
Cercopidae	*Cercopis intermedia*, *C. sanguinolenta*, *C. vulnerata*	Spain, UK
Cicadellidae	*Anoterostemma ivanoffi*, *Cicada orni*, *Cicadella lasiocarpae*, *C. viridis*, *Evacanthus acuminatus*, *E. interruptus*, *Graphocephala fennahi*, *Ledra aurita*; phloem feeders in the genus *Euscelis*: *E. incisus*, *E. lineolatus*, *E. ohausi*, *E. venosus*	France, Spain, UK
Cicadidae	*Cicadatra atra*, *Cicadetta fangoana*, *C. montana*, *Cicadivetta tibialis*, *Lyristes plebejus*, *Tibicina corsica ssp. Corsica*, *T. haematodes*	France, UK

6. Symptoms of diseases caused by *Xylella fastidiosa* worldwide

Symptoms associated with the presence of *Xf* depend on the specific combination between the host plant species and the bacterium strain. In some host–strain combinations, *Xylella* infection does not cause symptoms (non-expressed infections), and the infected host plants act as potential sources of bacterial inoculum.

The association between the manifestation of Olive Quick Decline Syndrome or OQDS symptoms in olive trees (Figure 2) and, the infection presented by *Xf* subsp. *pauca* belonging to different strains of the pathogen, has required a larger number of tests and analysis of the samples collected from symptomatic and asymptomatic trees. Plants tested positive for *Xf* from infected areas and showing no symptoms could be due to the existence of tolerance olive tree cultivars, and/or the appearance of a lag between the infection and the emergence of disease symptoms. Symptoms in olive trees negatives for *Xylella* presence would be associated with different pathologies (e.g. mycoses) causing crown wilt and other symptoms.

As bacteria invade xylem vessels, it blocks the transport of mineral nutrients and water. Symptoms associated with *Xf* infection has been related to water stress conditions, production of extracellular bacterial polysaccharide, and biofilm formation and cell aggregation. Symptoms consist of typical so-called burning, scalding, foliar scorched or marginal necrosis, foliage withering, defoliation, chlorosis or tanning in the margin of the leaf and dwarfing of the plant (see Figure 2).

Figure 2. (A – E) Symptoms of *Xylella fastidiosa* on olive trees in Puglia (Southern Italy); (F) Symptoms of OQDS caused by *Xf* subsp. *pauca* ST53. (A – E) Boscia, D., Nigro, F. and Guario, A.; (F) Landa, B. B. and Navas-Cortés, J. A. (2017)

Source: EPPO,https://www.eppo.int/ACTIVITIES/plant_quarantine/shortnotes_qps/shortnotes_xylella

The ALS disease is characterized by chlorosis and leaf scorching symptoms starting from terminal leaves evolving to widespread dry foliage of the tree. The CVC symptoms are described as chlorosis on the leaves – which small yellow punctiform decolouration – and decay of trees, making them economically unproductive. In the case of the PPD, no symptoms are noticed at the leaf level (does not cause leaf scorching) and death of the trees. However, the disease induces abnormal growth of the tree and decreases the amount of fruit produced.

7. Economic, social and environmental consequences as a global threat in Europe

Xf is a quarantine bacterium in the European Union (EU) since 2000, according to Directive 2000/29/EC, and is also included in the list A1 of the European and Mediterranean Plant Protection Organization (EPPO). It is now considered as a serious risk that threatens several crops and agricultural products of great strategic importance all over the world and, for which no efficient and sustainable methods for its integrated control is to be had.

Since the first detection of *Xf* in Apulia, the disease has spread throughout the region, causing enormous economic and environmental losses and affecting numerous cultivated and wild plant species. At the end of 2014, the entire province of Lecce, in the Puglia region, was declared an infected area and only containment measures are applied. The presence of *Xff* in Europe, and specifically in the wine regions of the continent, could be considered a great risk for the European agricultural economy with the death of entire vineyards in few years. The pathogen subspecies *multiplex* and *fastidiosa* are now threatening the most important European almond production area, putting at risk the fruit producers from Spain and Italy.

8. Current worldwide distribution of *Xylella fastidiosa*, the presence of the disease in the European Union

Xf is wide distributed throughout the American continent. In North America, *Xf* has been detected in Canada, Mexico and US; in Central America and the Caribbean, in Costa Rica and Mexico; and in South America, in Argentina, Brazil, Ecuador, Paraguay and Venezuela.

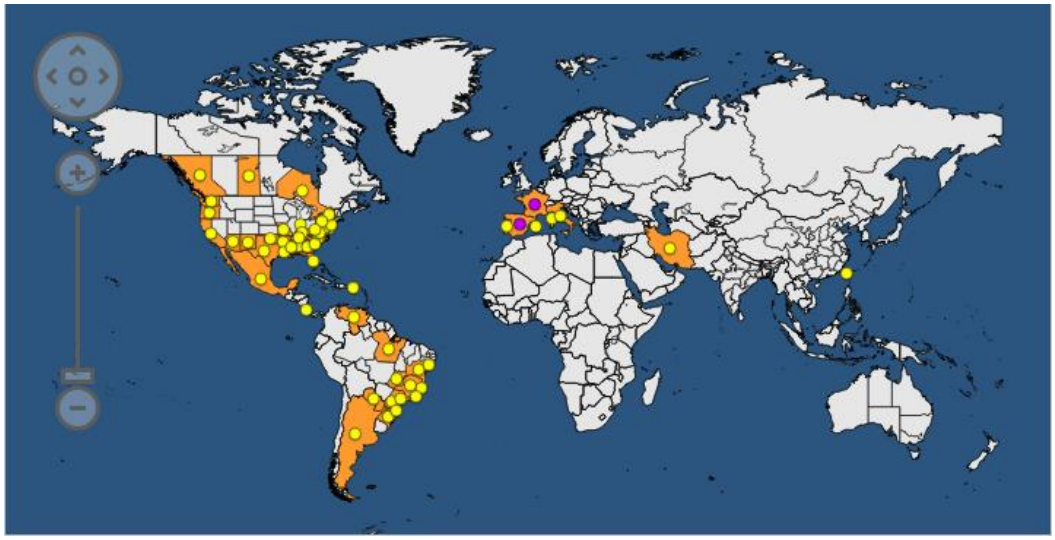

Figure 3. Geographical distribution (world map) of *Xylella fastidiosa*
Source: EPPO (updated on 31 January 2019); https://gd.eppo.int/taxon/XYLEFA/distribution

Official surveys carried out by EU Member States confirm so far that its presence is limited to Italy (Southern Apulia and Northern Tuscany), France (Provence-Alpes-Côte d'Azur and Corsica), Spain (Balearic Islands, Valencian Community, Madrid, and Andalusia), Portugal, and an isolated case in a greenhouse in Germany (Saxony). Outside of America and Europe, *Xf* has been detected in Iran thus revealing that it is moving to Asia.

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Chapter II. Best practices to increase resilience

1. Efficient use of water. Irrigation scheduling methods

Efficient irrigation is a crucial water management objective under scare water supply, as well as under climate uncertainty and variability. Plants require water for health growth, high yielding and high-quality production. The main goal of irrigation scheduling is to define the adequate amounts of water to apply to cropped fields with the proper irrigation timing, frequency and time duration to avoid the occurrence of water stress during the crop cycle.

There are many alternatives to schedule irrigation for most efficient use of water and to optimize production. The commonly used **irrigation scheduling methods** are:

a) Crop Evapotranspiration (ET) Scheduling.

Evapotranspiration (ET) is the loss of water to the atmosphere through the combined processes of water evaporation from the soil and plant surfaces (E) and transpiration through the plant tissues (T). Both processes occur simultaneously and are difficult to determine separately (Allen et al., 1998).

Crop ET is estimated from historical ET or Real-time ET information (Weather station) following:

$$ETc = ETo \times Kc$$

where ETc is the Crop Evapotranspiration (mm per day), ETo is the reference evapotranspiration (mm per day) (using historical ET data or weather station) and Kc is the crop coefficient (depend on crop stage).

The crop water needs can be calculated from the soil-water balance in the root zone using the equation presented below:

$$I_n = ET_C - P - G_w - \Delta SW + RO + D_p$$

where I_n is the net irrigation (mm), ET_c is the crop evapotranspiration (mm), P is the total precipitation (mm), G_W is the capillary rise of water (mm), ΔSW is the change in soil water storage in the crop root zone (mm), RO is the surface runoff (mm) and D_p is the deep percolation from the root zone.

This equation can be simplified where groundwater aquifers are deep and there is no shallow watertable to the following equation (Brouwer & Heibloem, 1986):

$$I_n = ET_C - P_e$$

where P_e is the effective precipitation is the fraction of rainfall that infiltrates and stores in the soil root zone and available to plants (mm).

The gross irrigation depth can be calculated accounting for water losses due to irrigation inefficiencies, which can be all included in a term called water application efficiency, as shown in Equation 9.

$$I_g = \frac{I_n}{AE}$$

where I_g is the gross irrigation requirement (mm) and AE represents the application efficiency which depend on the irrigation system.

The maximum net irrigation depth to apply during an irrigation event depends on the available soil water holding capacity (AWC), the depth of the root zone (Zr) and the Management Allowable Depletion (MAD).

The time-duration necessary to apply the gross irrigation depth for microirrigation systems depends on the system capacity.

b) <u>Soil-Based Method</u>

Soil moisture monitoring system consist of sensors that measure the current soil water status in the root zone during and between irrigation event. Irrigation scheduling based on soil moistures sensors data entails the following steps:

- ✓ Observe soil moisture data frequently
- ✓ Start irrigation at specific levels of soil moisture (allowable depletion, allowable matric potential or tension)
- ✓ Stop irrigation when soil moisture reaches target levels
- ✓ Predict the next irrigation based on the measured soil moisture depletion rate.

Soil moisture can be measured in terms of soil water content and tension. The selection of a device should be based on evaluating the sensors' advantages and disadvantages in term of cost, installation, ease of use, read-out and logging, and maintenance needs.

c) Plant-Based Irrigation Scheduling

Plant water-based monitoring is another approach used for irrigation scheduling, alone or in combination with crop ET. Water status in plant tissues directly affects metabolic and physiologic processes. Plant water status provides information about how water moves through the soil-plant system and about atmospheric evaporative demand.

Numerous methods have been developed to measure or monitor parameters directly or indirectly related to plant water status (Hsiao, 1990). Some of them are listed below:

- ✓ Plant water potential
- ✓ Relative water content
- ✓ Hydraulic press
- ✓ Organ dimensions
- ✓ Stomatal opening
- ✓ Canopy temperature
- ✓ Xylem cavitation
- ✓ Expansive growth of leaves or stems

The choice of a specific measurement method depends on the plant's relative sensitivity to water deficit and on the particular purpose of the measurement. The most common parameters measured in the field are plant water potential and canopy temperature.

These three irrigation scheduling methods such as the combinations of these approaches are feasible to establish irrigation scheduling on the demonstration farms.

1.1. Irrigation strategy

The irrigation strategy is an aspect to consider in irrigation scheduling. The irrigation scheduling approach most frequently used aims at **full satisfaction of the crop water requirements**, which usually results in maximum crop growth and yield, and stand longevity. However, different irrigation strategies can be implemented to maximize the net income for farmers based on the availability and cost of the different production factors and on the crop yield response to water.

Partial irrigation strategies may be considered and pursued during periods of limited water supply or to achieve specific production quality targets. Regulated and Sustained Deficit Irrigation are common scheduling approaches for partial irrigation that could be used to achieve specific targets for crop production or to maximize water-use efficiency and water productivity (more crop per drop).

- Strategy 1: Sustained Deficit Irrigation, Implementation of deficit irrigation during the entire crop season by applying a specific fraction of the water needed (ETc) for achieving the maximum yield.

- Strategy 2: Regulated Deficit Irrigation. Full irrigation applied during some crop development stages as well as a specific percentage of full ETc is applied during other phases of the crop growth.

Different crops have different sensitivities and tolerances to water deficit during their different growth and production stages. A good understanding of the crop's yield responses to water is needed to successfully implement a partial irrigation strategy.

Several research studies have been conducted on partial irrigation strategies with successfully results in olive and almond trees. In these studies, yield was maintained and production quality improved in some cases, while the amount of applied water and energy were substantially reduced.

In the demonstration farms, an irrigation specialist will determinate which partial irrigation scheduling must be applied in each crop adapted to the crop development stages, climatic condition of each year and water available.

2. Soil and plant health

2.1. Biota and soil health

The living organisms in soil are called soil biota. The biota of the soil is composed of all the flora and fauna of the soil. The great majority of the organisms that live in soil are found in soil superficial layers, where the conditions of humidity, temperature, ventilation and luminosity, as well as the available space allow these organisms to complete their life cycle. The organisms that inhabit soil participate actively in the nutrients cycle such as nitrogen cycle, carbon, phosphorus, potassium, etc. In addition, they regulate soil organic matter dynamics, carbon sequestration and greenhouse gases emission. In this way, the organisms that live in the soil modify the soil physical structure and therefore its water retention capacity, and the amount and availability of nutrients for the crop.

Soil exploitation has allowed the growth and development of human population, but intensive agriculture and monoculture have damaged soil quality and reduced its

fertility. Soil is one of the most diverse habitats on the earth, containing thousands of organisms, but this diversity has been altered by agricultural practices that have had a great impact on soil biota. In this sense, agriculture has a recognized impact on soil health and therefore on the organisms that inhabit it. Modern agricultural practices such as tillage and pesticides addition have replaced soil biological functions increasing external inputs dependence in order to maintain soil productivity. A good example of this is the modification of the relationship between bacteria and fungi in soil, which is affected by the addition of fertilizers and pesticides. In addition, there are organisms vulnerable to changes in soil pH. All these changes finally lead to an alteration in soil C/N (carbon-nitrogen) ratio. In addition, soil tillage reduces the hyphae fungal number when soil aggregates break. The key in soil management is to use agricultural practices to positively influence health and soil fertility. But this involves understanding and restoring microorganism populations, in order to obtain a beneficial effect on crop productivity. Microorganism populations and the processes associated with these populations influence soil fertility and structure, affecting crop productivity in various ways. Fortunately, respectful and technologically advanced agricultural practices are being developed, which in fact can improve in some cases severely damaged soils through the incorporation of soil microorganisms.

On the other hand, soil biology encompasses not only soil organism's biomass (quantity) but also soil organism's activities. Soil microorganisms are present in astounding amounts, although individuals may not be visible to the naked eye. For example, it is estimated that there are at least one billion bacterial cells per gram of soil distributed between thousands and millions of individual species. It has been calculated that soil microbial biomass can approximate the sum of all living biomass on earth surface. In soil microbial communities, maintaining critical functions may be more important than maintaining taxonomic diversity. An essential soil microorganism function is nutrients processing and recovery, which leads to the accumulation of soil organic matter and hence maintenance soil fertility. This often requires extracellular enzymes activity whose function is to transform complex organic compounds into simpler compounds (sugars, amino acids, ammonium, phosphates) in order to facilitate their

assimilation. Soil enzymatic activities have been related to soil physic-chemical characteristics, microbial community structure, predominant vegetation, etc. In turn, estimating soil enzymatic activity gives an idea of the processes that occur in the soil. For example, β-glucosidase activity reflects the state of organic matter decomposition. β-glucosidase belongs to the group of enzymes that catalyse hydrolytic conversion of cellulose to glucose, soil microorganisms food source. In addition, soil enzymatic activity is useful to determine soil degradation. In this sense, catalase activity is related to soil degradation, since this enzyme is one of the global indicators of the state of the soil. This enzyme is necessary to break down the hydrogen peroxide produced during cellular metabolism and is one of the most abundant enzymes in nature. In turn, through soil enzymatic activity the state of certain soil plant nutrients can be determined. This is possible for example by measuring soil urease and phosphatase activity. Extracellular phosphatases released not only by soil microorganisms but also by plants roots. In turn soil free enzymes could retain their properties once microorganism from which they come is dead. Invertebrates such as earthworms also release phosphorus from organic phosphorus. However, it is assumed that the soil enzymes come mainly from microorganisms that inhabit soil. Therefore, soil microbiota seems to be the most logical option to supply most of these soil enzymes. The reason lies in the significant amount of biomass and the high metabolic activity that develops in short time, which allows them to produce and release relatively large amounts of extracellular enzymes with respect to what would come from plants and animals.

Among the organisms that inhabit soil we find soil bacteria. Soil bacteria are the smallest and most numerous soil microorganisms. Most of them are heterotrophs and participate in organic matter decomposition processes and energy and nutrients recycling; such as nitrogen, phosphorus, sulphur, iron and magnesium. Some bacteria are able to use atmospheric nitrogen, which can be used by plants when bacteria die, thus participating in nitrogen cycle. Some bacteria also produce antibiotics and toxins for other soil organisms, as well as animal and plant pathogens.

The bacteria that inhabit the soil are found in a much higher density in the area of the soil that is in direct contact with plant's roots. This area of the soil has been called

rhizosphere. Roots produce exudates, which attract microorganisms from soil root surface. The relationships established between plant and soil microorganisms could positively affect plant when symbiosis is established with beneficial microorganisms or could be negative when soil pathogens exert their action on roots. The rhizosphere is a zone of dynamic interaction between plant root and soil microorganisms, characterized by an increase in microorganism biomass and its activity. Thus, the number of bacteria that inhabit the rhizosphere is between 10 and 1000 fold higher than the number of bacteria present in non-rhizospheric soil (farthest from the root). In this way, rhizosphere provides a complex and dynamic microenvironment for roots crop and organisms that inhabit the surrounding soil. The production of radical exudates attracts surrounding microorganisms, being able to establish harmful relations with plants, in the case of pathogenic microorganisms, and beneficial ones; this is the case of the bacteria that promote plant growth (PGPR).

Plant growth promoting rhizobacteria, from now on PGPR, are those bacteria that inhabit rhizosphere and can promote growth through hormones or stimulating their immune system. According to the type of interaction established with the plant, PGPR can be classified as symbiotic or free-living bacteria. Most symbiotic bacteria live in the intercellular spaces of host plant, but some of them can establish mutualistic relationships with the host by penetrating plant cells. One of the best-studied examples so far is the symbiosis that is established between plant and Rhizobium bacteria, in this case, plant secrete flavonoids that act as a signal for bacteria to produce nodulation factors. Nodulation factors are perceived by plant root hairs inducing radical nodule formation. In these nodules, rhizobia could fix atmospheric nitrogen. This symbiosis benefits plants in nitrogen-poor soil, where nitrogen becomes a limiting factor for plant development, thus promoting growth. Several mechanisms to promote plant growth have been described through free-living rhizobacteria in order to promote plant growth. Many species of free-living soil microorganisms have the capacity to fix nitrogen or increase nutrients availability through siderophores and organic acids production. In addition, they modulate plant regulatory mechanisms through hormones production or other compounds that can influence plant development. In general, plant growth can be stimulated by PGPR

bacteria directly; due to nutrient availability (nitrogen, phosphorus, potassium and essential minerals) and hormones or indirectly (Table 1); through inhibitory effects on soil pathogens.

Table 4. Mechanisms through PGPR bacteria promotes plant growth

Indirect Mechanisms	Direct Mechanisms
Antibiotic production	Atmospheric nitrogen fixation
Lytic enzyme production	Phosphorus solubilisation
Resistance induction	Potassium solubilisation
	Siderophore production
	Plant hormone production

A) Indirect mechanism through PGPR promotes plant growth:

- ✓ Antibiotic production:

Antibiotics production by PGPR bacteria is considered one of the most powerful biocontrol mechanisms against pathogens. A large variety of antibiotics have been identified produced by bacteria of the genera Pseudomonas, Bacillus, Streptomyces y Stenotrophomonas.

- ✓ Lytic enzyme production:

PGPR bacteria of the genera Pseudomonas and Azotobacter produce lytic enzymes such as chitinases, dehydrogenases, beta-glucanases, lipases, proteases, etc. Through this enzymatic activity they play an important role in promoting plant growth of indirectly, protecting the plant from both biotic and abiotic stresses by suppressing pathogenic fungi growth.

- ✓ Resistance induction:

Interactions between plant roots and PGPR bacteria can result in resistance induction to different plants pathogens. This phenomenon has been called Systemic Induced Resistance (ISR). This type of resistance has similar characteristics to human

defence system, where a substance produced by a pathogen can trigger in plant reactions that lead, for example, to increase enzymes production that will act against a specific pathogen. Good examples of PGPR bacteria, which produce these reactions, are Bacillus and Pseudomonas.

B) Direct mechanism:

- ✓ Atmospheric nitrogen fixation:

Nitrogen is an essential element for all life forms and is a vital nutrient for plant growth and development. The atmosphere contains about 78% nitrogen, but the plants cannot fix atmospheric nitrogen.

PGRP fix atmospheric nitrogen through nitrogenase enzyme. This can occur through two pathways, one of them is radical nodules formation described above or being fixed by free-living bacteria from the genus Azoarcus, Azotobacter, Acetobacter, Azospirillum, Burkholderia, Diazotrophicus, Enterobacter, Gluconacetobacter, Pseudomonas and Cyanobacteria.

- ✓ Phosphorus solubilisation:

Phosphorus is one of the most important elements in plant nutrition after nitrogen. This element plays an essential role in most of the plant metabolic processes such as photosynthesis, energy transfer, signal translation, macromolecule synthesis and respiration. Although phosphorus is found abundantly in soil, plants are unable to use it because 95-99% of soil phosphate is insoluble, immobilized or precipitated. Plants can absorb phosphates only in the forms of H2PO4 and HPO2-. Pseudomonas, Rhizobium and Bacillus are among the most powerful phosphate solubilizers. The main solubilisation phosphate mechanism is the production of organic acids and acid phosphatases. For example, phosphorus-solubilizing bacteria (P. putida) solubilize mineral phosphorus and, through the hydrolytic action of its phosphatase enzymes, mineralize organic phosphorus.

- ✓ Potassium solubilisation:

Potassium is the third most important nutrient after nitrogen and phosphorus in plant nutrition. Generally, soil potassium concentration is usually very low because more than 90% of soil potassium is found as insoluble rocks or forming silicates. In addition, due to incorrect soil fertilization, potassium deficiency is usually one of the biggest problems for agricultural production. Potassium insufficient amounts cause poor root development, slower plant growth, smaller seeds and low crop yield. Pseudomonas, Burkholderia, Acidothiobacillus ferrooxidans, Bacillus mucilaginosus, Bacillus edaphicus, Bacillus circulans and Paenobacillus sp. are able to solubilize potassium from soil mineral potassium.

- ✓ Siderophore:

Iron is an essential micronutrient not only for plants but also for microorganisms and fungi. Although iron is one of the most abundant elements in the earth's crust, it is in the form of ferric ion (Fe3+) which is very poorly soluble, therefore the assimilation of iron by living organisms is extremely low. Microorganisms have developed mechanisms for the assimilation of iron, these mechanisms include the production of siderophores, which are low molecular weight molecules capable of transporting iron into the cell. In this way, PGPR bacteria of the genus Aeromonas, Azadiractha, Azotobacter, Bacillus, Burkholderia, Pseudomonas, Rhizobium, Serratia and Streptomyces are able to supply iron to the plant. In addition, indirectly, in acid soils with low iron concentrations, they suppress the growth of pathogens due to the fact that they sequester available iron that cannot be used by these pathogens for their proliferation. Recent studies have shown that Pseudomonas spp produces the suppression of pathogenic fungi that inhabit the soil through the release of iron chelating siderophores. One example is the siderophore produced by strains of P. putida, which intervenes in the suppression of the phytopathogenic fungus Fusarium oxysporium in iron-deficient soils; this suppression is lost when the soil is replenished with iron, a condition that suppresses the production of iron chelators by siderophore producing microorganisms.

- ✓ Hormones production:

PGPR bacteria produce plant hormones such as auxins, gibberellins, cytokinins, and ethylene, being able to modify root cell proliferation. Changes in root cells proliferation lead to modification of root architecture due to lateral roots and radical hairs overproduction, these mechanisms increase root water and nutrients absorption.

Indoleacetic acid is one of the most common plant natural auxins, this hormone among other things, stimulates root growth. In addition, within the plant it affects cell division, growth and differentiation, stimulates seed germination and tubers and controls vegetative growth processes, among others. Furthermore, it affects photosynthesis, pigment formation and stress resistance. Tryptophan is an amino acid that is frequently found in root exudates. It has been identified as the precursor molecule in indoleacetic acid biosynthesis in Pseudomonas, Rhizobium, Bradyrhizobium, Agrobacterium, Enterobacter and Klebsiella.

Gibberellins regulate different plants processes such as seed germination, stem elongation, flowering and fruiting. Numerous studies have shown that Azospirillum spp produces gibberellins and these produce plant changes as a promotion in root growth and a germination rate increase.

Cytokinins regulate cell division and differentiation in plants meristematic tissues. There are two structural groups of cytokinins: those of the adenine type such as kinetin, zeatin and 6-benzylaminopurine and phenylurea type such as thidiazuron. These hormones have been associated with many physiological and cellular processes, including senescence delay due to an accumulation of chlorophyll and organs formation in many different tissues, root and root hairs development, the root elongation, stem initiation and leaf expansion. In addition, they promote sprouting buds in fruit trees. At least 90% of the bacteria isolated from the rhizosphere are capable of producing compounds that have cytokinin activity. A good example of this is Azospirillum brasilense, which produces isopentyladenine, isopentyladenine ribose and Zeatin.

2.2. *Induced systemic resistance in plants*

Plants establish multiple levels of defence responses, including physical barriers such as cuticle and cell wall, as well as chemical defences, such as antimicrobial or anti-insect compounds. The first plant defence barrier is cuticle. To avoid phytophagous attack it has been observed substances production that triggers cuticle hardening as a consequence of pathogens attacks. A large percentage of plant pathogens are biotrophs that require compounds from living host cells. Recognition of pathogen attack triggers a hypersensitive reaction (HR) in the plant, which includes generation of reactive oxygen intermediates (ROIs). In addition, apoptosis or programmed cell death is triggered. In this way, the supply of nutrients to the cell attacked by pathogens is interrupted, which causes pathogen death through starvation. This type of response is not effective against pathogens that feed on necrotic tissue such as Botrytis cinerea or Sclerotinia sclerotiorum.

On the other hand, induced resistance (ISR) is caused by PGPR soil microorganisms and depends on jasmonic acid pathway. While acquired systemic resistance (SAR) is caused by different organisms and depends on salicylic acid pathway. In the case of ISR, it has been recognized as an attractive tool for the management of plant diseases in modern agriculture.

Acquired systemic resistance (SAR) was first observed in the upper leaves of tobacco plants after tobacco mosaic virus (TMV) was inoculated into the lower leaves of the same tobacco plants. Decades of research have identified two common characteristics to the SAR response in several different plant species: (1) broad-spectrum effectiveness against diverse pathogens and (2) a long-lasting effect following elicitation. During SAR responses caused by necrotrophic pathogens, plants obtain systemic resistance against not only the inducing pathogen but also different pathogens. For example, SAR response caused by TMV was not limited to TMV but was effective against four different plant viruses and even fungal pathogens. Once the SAR response was elicited, the response was effective for more than 20 days. It is evident that this type of defence response is of great interest for plant pathogens management in the field, due to its low aggressiveness

with the environment. It is probable that thanks to all these characteristics, different chemical compounds have been developed commercially that produce in the plant the same SAR response that originates when the plant is attacked by a pathogen. One of the great advantages of using this type of compound is that the harmful effects for human health or the environment are minimal and, in some cases, null. However, the use of this type of substances can cause a negative effect on the growth of plants. This phenomenon is known as an "allocation fitness cost" or "trade-off", since a substantial amount of metabolic resources is needed for the manifestation of defence responses in the plant in response to chemical substances, resulting in a decrease in growth due to lack of energy. The first experiments to obtain SAR responses artificially revealed that low concentrations of SA (salicylic acid) did not activate plant resistance but increased the expression of defence-related genes.

Similar to the SAR response, colonization of the root by certain rhizobacteria induces a systemic resistance response that is effective against plant pathogens. The term ISR describes "the activation of the physical or chemical defences of the host plant by an inducing agent". Interestingly, PGPR bacteria induce an ISR response and promote plant growth at the same time by providing hormones, antibiotics, etc., as discussed above. This is a promising way to overcome physical cost allocation conditioning (acclimation) in order to obtain optimum crop performance while a potential disease is reduced. In this sense, PGPR bacteria are already being used as microbial inoculants to control plant pathogens and promote their growth.

On the other hand, a great advantage that presents the use of substances that trigger a defence response in the plant is the case of production of defence compounds such as phenolic compounds. In order to protect their own tissues, plants have developed efficient antioxidant systems. It includes both antioxidant enzymes, which catalyse the decomposition of ROS, as well as non-enzymatic antioxidants such as ascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), carotenoids and numerous phenolic compounds. The non-enzymatic antioxidant compounds allow the plant to capture toxic oxygen species that are produced in the responses to pathogen attacks neutralizing them. In the specific case of the olive tree, the production of phenolic compounds increases oil

quality. Polyphenols are important for flavour and oil stability. In addition, high polyphenol content seems to be beneficial for oil shelf life and stability correlates well with total oil phenols content. Various phenolic compounds were tested for their antioxidant effect; it was found that hydroxytyrosol is the most potent and more effective than butylated hydroxytoluene (BHT). The aglycones are also highly correlated with the stability of the oil. In the case of the almond, the phenolic compounds are concentrated in the skin, and also have a high antioxidant power. With which, an increase in phenolic compounds in the skin of the almond would provide an advantage at the nutraceutical level at the time of its commercialization.

3. Auxiliary flora and fauna

3.1. The cover crops

Cover crops are types or forms of vegetation that cover an area of soil of the crops. This protection is extremely important for the environment, because it works as a kind of roof and protects the soil against erosion, protects our fauna and flora and balances the temperature.

3.2. Effects of a cover crop

A cover crop that has in its constitution Leguminous species, allows on average these legumes to fix between 50 to 220 kg / ha of Nitrogen in the soil - depending on the texture, pH, temperature, soil, and soil moisture.

- Biodiversity

Soil living matter plays a key role in maintaining biodiversity.

Soil microorganisms are important for the decomposition of organic matter, which increases the availability of nutrients to plants.

A suitable cover crop allows to increase soil temperature stability, and also to increase soil moisture, thus creating a more favorable environment for the development and multiplication of these beneficial microorganisms (Auxiliary Fauna).

- Erosion Control

The most effective way to control soil erosion is to improve its structure. By improving soil structure, there is an increased rate of infiltration of water into the soil.

By increasing the infiltration rate, the amount of water available in depth for the plants also increases.

- Auxiliary Fauna

The increase of biodiversity creates favorable conditions for the activity of the auxiliary organisms.

Populations of natural enemies of pests (auxiliary organisms) tend to increase due to increased availability of food and alternative habitats that create conditions more favorable to their survival and reproduction.

With helpers nearby, plants become less attractive to pests.

3.2.1. Visible effects on soil

- Weed Control (minimizes the use of herbicides);
- Protection against soil erosion;
- Increase in the level of organic matter;
- Prevents leaching of nutrients;
- Improvement of soil structure;
- It improves the movement of the machines during periods of precipitation; and
- Decrease in soil compaction.

3.2.2. Visible effects on the plant

- Increased root depth (through improved soil structure);
- Decrease of damage to the roots of the plants (avoiding the passages of tools of soil mobilization); and
- Increased Biodiversity that contributes to the reduction of the incidence of pests and diseases (by increase of the auxiliary microorganisms).

3.3. The hedges

Any structure composed of herbaceous, shrub or arboreal vegetation, arranged in strip, whose function is to divide, seal or protect the property in relation to the action of the wind is called hedge.

Hedges are structures that have several purposes, such as: wood production, protection against frost, erosion and wind, water protection and biodiversity balance.

Species that constitute a hedge affect its structure and, consequently, its functions, in particular as regards performance as a potential "habitat" of fauna and flora. Regarding the composition, the hedges are divided into simple, when constituted by a single plant species, or mixed, when in its composition are several species.

Hedges should be integrated and properly articulated with the forest and agricultural landscape and preferably occupy areas of low productive potential, such as steep slopes, cultivated plot boundaries, rural roads and bordering agricultural holdings.

These ecological infrastructures should preferably be installed perpendicular to the dominant wind direction, bearing in mind that the N-S orientation minimizes shading.

The hedges, when installed, aim essentially: to avoid the mechanical action of the winds; avoid damages due to thermal extremes (cold, hot or dry winds); decrease wind and water erosion; and avoid the damage resulting from marine aerosols in coastal areas. The primary effect of any hedge system, whether natural or artificial, is to reduce the

wind speed, which may reach 30 to 50% lower depending on the characteristics and constitution.

The capacity of a given hedge to house natural enemies varies with the farm system, the location of the crop plot on the farm, the time of year and the floristic composition and phenology of the remaining vegetation.

3.3.1. Hedge effects

The advantages of introducing hedgerows in agricultural activity are as follows:

- Protect the winds
- They confer privacy
- Protect from noise
- Conferencing security
- Promote and conserve biodiversity

3.4. Species and cultivars with attractive potential of Xylella fastidiosa auxiliary insects

Whether in the interior space of the crop or in the surrounding space, vegetation, whether in the form of hedge or vegetation cover, can play a positive role in the proper maintenance of biodiversity.

Next, we describe some Shrub, Arboreal and Herbaceous plants with attractive potential of insect predators of *Xf* vector insects:

Table 5. Arboreal and herbaceous plant with attractive potential of insect predators to *Xf* vector insect

	Scientific name
Trees and shrubs	*Viburnum tinus, Crataegus monogyna, Arbutus unedo, Prunus spinosa, Punica glutinosa*
Herbaceous	*Mentha suaveolens, Urtica dioica, Pastinaca sativa, Matrichania chamomilla, Brassica napus, Chrysanthemum coronarium, Hypericum perforatum, Lupinus luteus, Ervilhaca sativa, Centaurea cyanus, Borago officinalis, Calendula arvensis, Phacelia tanacetifolia, Anethum graveolens, Helianthus annuus*

3.5. Sowing and planting

3.5.1. Covered vegetable

In planting sowing, it is necessary to take into account some factors that can be decisive for a correct installation, since it is a permanent crop (for several years), it is essential to master the technique associated with the sowing process, as it can determine success in your installation.

- *Soil Preparation* -

Whatever the Seed Mixture to be installed, good soil preparation is essential for sowing, so it is necessary that the soil is thoroughly ground in a layer of 10 to 15 cm (the clods are enemies of a good sowing).

- *Plant Density* -

The species to be installed must be adjusted for each sowing situation.

Because they are seeds of very small size, sometimes less than 1 mm in diameter, the density of plants is much lower than what most farmers are accustomed to.

Leguminous seeds should be inoculated in order to ensure a high fixation of atmospheric nitrogen.

- *Seed Depth* -

There is a universal rule in the agricultural world, which determines that the depth of deposition of a seed in the soil must take into account its size.

Therefore, because the seeds are very small, the depth of sowing should not exceed 1cm, and should be accompanied by a roll (in order to improve the contact between the seed and the soil).

- *Correction of Fertility* -

Correcting soil fertility is usually a necessary operation. Thus, it is fundamental to perform a soil analysis, the values obtained in it being important to determine which or which nutrients are missing / excess.

We know that soils of very low pH (4.0 - 5.0) must be corrected with dolomitic limestone (Calcium Carbonate + Magnesium Carbonate) at the rate of 1,000 to 3,000 kg / ha. Correction of pH becomes a fundamental factor to take into account, because acidic soils do not allow the absorption of phosphorus by plants, especially by legumes.

Phosphorus presents as the "legume engine" because it is a fundamental element to its proper functioning, and to establish the symbiotic relationship with the Rhizobium bacterium in order to allow the fixation of the atmospheric nitrogen, transforming it into assimilable nitrogen for the plant.

It is important to monitor the level of soil fertility so as to intervene with the necessary corrections, thus ensuring a good maintenance of production per hectare.

- *Management of Plant Cover* -

In terms of the management of Covered Vegetation, there are two cutting times of the vegetation cover:

- 1st Period of Cutting * - before the beginning of flowering (in order to eliminate weeds and that are not of interest for the vegetal cover);

* cut not less than 10 cm in height;

- 2nd Cutting Season - after the plant has dried (after the seed is formed) to facilitate regrowth the following year;

For the success of Covered Plant it is fundamental, in the first year, to create a good seed bank, so that the species continue to germinate during the following years.

This ensures the longevity of Covered Vegetable.

3.5.2. Hedges

To plant a hedge, one must open a trench of 0.50 m in depth and the length that is necessary, aerate the earth and incorporate an organic corrective and a bottom fertilizer.

It is important to water 2 or 3 times a week during the first year of planting. The shrubs are arranged in a straight line, in a single line, if you wish to create a compact hedge. On the contrary, if more space is available, the hedge can become larger by planting two rows of parallel plants arranged alternately.

The planting distance depends on the type of plant although it is usually about 30 to 80 cm for the shrubs and 2.50 m for the large trees that are used as windswept hedges.

- *Management and management of hedges* -

The most important maintenance operation in the hedges is pruning. This operation allows to maintain the hedge in the desired dimensions, reinforces and densifies it.

A pruning, one year after planting, will fill the base with shoots. This pruning is always carried out at the end of winter when there is no longer any risk of large frost. It consists of pruning the new shoots in half and must be done every year until the hedge reaches the desired height.

The goal is to maintain a certain harmony in the size of the shrub (to control those of fast growth and to favor those of slow growth).

Pruning stimulates the production of lateral branches that will give flowers, leaves and decorative branches the following year.

3.6. Auxiliary fauna

Auxiliary Fauna are organisms that oppose the development of the populations of their enemies, contributing or preventing them from causing harm to them.

For sustainable agriculture it is necessary to maintain or create a balanced environment with a diversified agro-ecosystem. In this perspective, it is very important to promote biodiversity within the diverse cultures as a way to provide ecosystem services that directly benefit the producer, especially with the natural limitation of the enemies of the crop, which in this case will be the vectors of Xylella fastidiosa and belonging to the Hemiptera Order , mainly Cicadelideos (Subfamily: Cicadellinae), Afroforídeos and Cercopídeos. Basically, all the insects that feed on the xylem.

As a way of controlling the population of the vector of Xylella fastidiosa it is very important to think of alternatives that may help in the control of its vectors, and it is in this context that the concept of Functional Biodiversity can become part of the solution, being the component of Biodiversity which can be used for the benefit of the crop, through Natural Limitation, where the auxiliary fauna plays a fundamental role.

3.6.1. Xylella fastidiosa auxiliary fauna

In order for the auxiliary fauna to be established on farms, it is essential to provide them with the basic conditions for their development and proliferation, and this can be done through the selection of the Ecological Infrastructures (IEEs). Obviously, this selection will have to be made based on the morphological characteristics of the auxiliary fauna, namely, to verify the compatibility between the type of flower available and the buccal reinforcement of the auxiliaries that can be most effective for the **Xylella** vectors.

One of the important IEEs is the crop coverings between the lines of crops, which as they are arranged on the ground, can be considered as Ecological Corridors, and as a rule, they represent about 80% of the biodiversity of a farm. In addition to these, the

existence of Hedges is also important because they allow the recolonization of the crop by auxiliaries.

Regarding the most important auxiliary fauna for the control of Xylella vector species, we can refer to:

Family Coccinellidae - are mostly predatory individuals in both the larval and adult stages. The design of the wings and the color combination varies from species to species and often within the species itself. They are characterized by being quite ravenous and have strong jaws that allow them to grind the insects they feed on;

Family Formicidae - Myrmica genus - In this genus can be found more than 160 species identified, in addition to several sub-species. They live mostly on the ground and feed on other insects. They have strong morphological structures capable of catching and feeding on adults of Cicadaidae;

Miridae family - they are heteroptera that are not normally present in large quantities due to their slower development, however after this phase occurs and establish themselves in the culture they become very effective predators.

Syrphidae Family - A characteristic of Syrphidae is their ability to remain in the air, almost without moving, flapping their wings very quickly and then making short flights, with which they skilfully escape predators. Adult syphidians are remarkably mobile, being able to rapidly colonize cultivated land, from places of refuge or from hibernation. The larvae of a large number of species of Diptera are among the most active predators of the agrarian ecosystem.

Family Sphecidae - a cosmopolitan family of wasps, usually dark in color, some with metallic reflections or bright colors, measure 20 to 40 mm. Your abdomen is large and with a petiole or a thin waist. They differ from bees in which hair is simple and not branched

There is also another group of important helpers in controlling vectors, which are Arachnids, where we find spiders. These are the most frequently observed predators in the agricultural ecosystem. For the most part they prey on insects and can use various

techniques to capture their prey: some build webs, others jump, run or hide in the environment in which they are inserted, using the surprise factor to hunt. Many species live on vegetation, while others live on the soil surface, preferring soils with plant residues. The shape of the body, the pattern of distribution of the eyes, the type of web that they elaborate and the behavior, are characteristics that contribute to the identification of the family to which it belongs.

Also important are hymenoptera of the Dryinidae Family (ectoparasitoids that attack nymphs and adults of the cicadelles) and the Family Mymaridae (endoparasitoids that attack the eggs) and the Family Encyrtidae, which are small wasps that move to the foam segregated by the vectors of Xylella and attack the nymphs who are there.

3.6.2. Measures contributing to the presence of auxiliary insects

The presence of natural enemies (auxiliaries), such as insects and birds, are an important means of regulating the growth of these same pests.

In fact, it is estimated that there are, on average, four species of auxiliaries for each species of enemy.

In the circumstances it is of great importance, on the one hand, not to destroy these useful organisms and, on the other hand, to enable them to reproduce and act effectively on the populations of harmful and harmful organisms.

There are several measures that can contribute to the presence of auxiliaries, such as: installation of live hedges consisting of shrubs; maintenance of spontaneous vegetation between the lines; and installation of aromatic plant lines.

The management of cover crops and adjacent vegetation, such as hedges, used as a useful refuge for insects, attract and sustain native populations of beneficial insects and is therefore a good solution. Selecting a natural enemy suitable for a specific pest target is very important. It is necessary to distinguish short-term application of natural animals from the long-term introduction of beneficial insects. Management of cover crops and adjacent vegetation, such as hedges, used as a useful refuge for insects, attracts and

sustains native populations of beneficial insects, being therefore a good solution. Selecting a natural enemy suitable for a specific pest target is very important. It is necessary to distinguish short-term application of natural animals from the long-term introduction of beneficial insects.

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Chapter III. Monitoring Activities

1. Protocol for monitoring and control procedures, sampling methodologies and data gathering

The impact during the Project is evaluated through the constant technical monitoring of Project Activities. Different indicators will be measured by specific methodologies (see Table 5). The parameters measured during the Project and after its completion will include in the following ten groups (Table 5):

I. Tree Health

I.1. Nutrient state through foliar analysis

This methodology is a technique that determines the content of nutrients in plant tissues sampled at a specific time or stage of development. Sampling requires rigor in order to determine plant health status, because the species, age, type of tissue and sampling time are variables that affect the interpretation of the results. We must take samples corresponding to similar tissues and in the same physiological state to those defined by the reference with which the results of the analysis will be compared, that is, following the instructions corresponding to the method of interpretation that the laboratory determine. Laboratory analysis will determine plant nutritional state such us nitrogen contain, C/N proportion, phosphorus concentration and macro/microelements.

I.2. Tree temperature

Xf cuts the flow of sap – water and mineral salts – within the plant, causing the temperature of the plant to rise which can be measured using a thermal camera. Images aerial via a drone or satellites will be analyzed. Georeferenced samples points could be required after the images processing.

I.3. Vegetative development

By obtaining, correcting and processing various bands of satellite images the vegetation situation will be evaluated, obtaining an evolution of the behavior of the same. For this, both historical satellite images contained in data banks will be used, as well as those that will be generated new over time during the period of application of this study.

The information obtained will allow to determine the NDVI vegetation index (Normalized Difference Vegetation Index) and to evaluate the vegetative development during the Project and the presence of physiopathy and diseases.

II. Soil quality

II.1. Available Water Capacity (AWC)

The AWC is the amount of soil water that is theoretically available for plants. This concept is based on the difference between soil field capacity – which is the maximum amount of water that soil can store –, and the wilting point of it – which is the minimum amount of water that soil can hold, and which is absorbable by the plants –. The AWC is different for each type of soil. Soil sampling and laboratory analysis are required. The sampling points are georeferenced in based on the types of soils identify by Map2Soil system (Greenfields by AGRODONE).

II.2. Physic-chemical analysis

Soil samples taken from georeferenced points established by the system of Map2Soil are analyzed in laboratory to determinate soil macronutrients, micronutrients, oligoelements, carbonates, % sand, clay and silt, and % O.M. (organic matter) oxidable, % C total and relation C/N.

II.3. Soil microbiological activity

Soil samples taken from georeferenced points are analyzed in laboratory to determinate fertility biological index and enzymatic activity such as, B-glucosidase, dehydrogenase, catalase, phosphodiesterase (phosphatase) and urease activities.

III.1. *Xylella fastidiosa* disease control

Plant tissues are sampled at a specific time or stage of development following the protocol of the laboratory (refer to Guidelines for the survey of *Xf* in the Union territory, European Commission, 2015).

Sampling of a plant and how to take the sample

The samples should consist of mature tissues (branches and leaves), collection of shoot portions in active growth should be avoided; mature leaves with petioles and woody twigs for perennial plant species; stem and the mature leaves in the case of herbaceous species, where possible.

How to preserve, transport samples

Samples should be shaken to ensure that no vectors are moved with the plant materials (e.g. adults vector will fly away, when the leaves, twigs are shaken). It is important to check that the sample does not contain any adult or juveniles of the vector species; put in closed container (e.g. plastic bag, etc.); kept at cool temperatures avoiding to expose samples for prolonged periods to sun or hot temperatures; transferred to the diagnostic laboratory as soon as possible, before the plant tissues deteriorate. In the laboratory, samples should be stored at 4 – 10 °C, and general principles of the good laboratory practices for analysis plant samples for bacterial infection should be followed.

III.2. Insect vectors trap

According to the diagnostic protocol adopted by the Standards Committee on behalf of the Commission on Phytosanitary Measures in August 2018 (see Annexes at the end of this document): "*Vectors should preferably be collected with sweeping nets (adults) or aspirators. Sampling for insects should preferably be carried out from late spring until early autumn to maximize the likelihood of detecting the bacterium*".

The demo site in Italy will trial a sound trap (nature vector control measures) – i.e.: vector mating call audios to sexually confuse and subsequently trap the vectors – that decreases insecticide use meaning fewer GHG (greenhouse gas) emissions.

In order to measure the effect of a certain hedgerow or cover crop on vector presence, a detailed map of hotspots will be made. On this basis Nutriprado (in all trial areas) will implement a vacuum technique and "pitfall traps" around the specific area which will collect all manner of insects in a fast and effective way. In problem areas further natural vector control measures (i.e. simple traps) will be placed, treating vector presence locally and effectively.

IV. Weather

IV.1. Weather conditions

Annual information on the parameters measured in public or private local climate stations, including: Temperature Maximum (ºC), Average Wind Speed (m/s), Temperature Time Maximum, Middle Wind Direction (º), Temperature Minimum (ºC), Radiation (MJ/m^2), Temperature Time Minimum, Precipitation (mm), Temperature Average (ºC), ET_0 (mm/day), Maximum Relative Humidity (%), Minimum Relative Humidity (%), Accumulated Precipitation (mm), Relative Medium Humidity (%), and Cumulative ET_0 (mm).

V. Quality

V.1. Organoleptic characteristics (olive oil)

The olive tree fruits and olive oil obtained will be evaluated annually to determine its quality by means of the following parameters: (i) content of fatty acids and volatile compounds present in green olives and their oil; (ii) total phenolic compounds; and (iii) sensory analysis.

The analysis of the volatile compounds present in olives or olive oil is carried out following a procedure in which a microextraction of headspace in solid phase is carried out. From a representative sample of olives or oil, the volatile compounds present are extracted by means of an automatic stirring for 15 minutes and at a controlled temperature

at 40 °C of a solution composed of the sample, salt and ultrapure water. In this analysis, a DVB / CAR / PDMS 50/30 µm fiber is exposed to the headspace. After sampling, the desorption of the volatile substances from the fiber coating is carried out at the injection port of the GC-MS for 3 min. All the compounds reported will be identified by 3 simultaneous methods: (1) retention rates, (2) GC-MS retention times (authentic chemicals) and (3) mass spectra (authentic chemicals and the collection of Wiley's spectral library).

V.2. Size and g USDA grade (almond)

The quality of the almond will be mainly evaluated by its size and USDA grades. Physical parameters: humidity, variety, twins, bitterness, mechanically damaged, and pieces (processed almond). Physical-chemical parameters: aflatoxins and pesticides. Microbiological parameters: *Listeria monocytogenes* and peroxide index.

VI. Water use

VI.1. Water use efficiency (WUE)

The WUE is the relation between water consumed (m^3) by the crop and water applied (m^3). Both values are calculated each year.

VI.2. Irrigation water productivity (IWP)

The IWP is the relation between annual yield (kg) and water applied (m^3). Both values are calculated each year.

VI.3. Stem water potential (SWP)

The SWP is measured by the Scholander pressure chamber on mature leaves to determine water status in olive trees during the growing season. The measures will be taken every week to schedule the irrigation following the Hydrosustainable Protocol (GALPAGRO).

VII. Carbon footprint

VII.1. CO_2 emitted during the agricultural processes

Annual CO_2 emitted in each farm will be estimated by Carbon Footprint Assessment ISO/TS 14067:2013.

VIII. Biodiversity

VIII.1. Auxiliary fauna

Occupancy rate of hotels nests and insect boxes can be determined by visual counting.

IX. Production value

IX.1. Money saved

This will be calculated by comparing production costs from the start of the Project to the end of the Project. Balance of production costs and income must be contributed by each farm.

X. Resilience of *Xylella fastidiosa*

X.1. Resilient Rate

New generation of olive trees will be tested for the presence of *Xf* bacterial DNA inside of them serving as a preliminary result in the spring/summer following its planting in the field. In the second year after planting (spring/summer) we will have definitive, visible results.

When, measure numbers and the partner responsible of each parameter are described in Table 5.

Table 5. Parameters to be measured for monitoring of the impact of the Project actions

Factor	Parameter	When	Amount in project	Partner responsible
(I) Tree Health	(01) Nutritional State (Foliar Analysis)	1 time a year: after full Bloom	5 strategies / Every demonstration (demo) site (Spain, Portugal and Italy)	GALPAGRO (SAHC, SALOV)
	(02) Tree Temperature	1 time a year: pre-harvest		AGRODONE
	(03) *Vegetative development* (NDVI, NDWI)	5 times a year: Blossoming, core hardening, oil production initiation, pre-harvest and post-harvest		
(II) Soil Quality	(04) Soil microbiological activity	1 time a year	Every demo site (Spain, Portugal and Italy)	AGRODRONE
	(05) Available Water Capacity (AWC)	First 6 months of the project (1-time project)		
	(06) Physicochemical analysis (SOM/SOC)	First and last 6 months of the project (2 times project)		
(III) Disease prevalence	(07) *Xylella fastidiosa* disease control	Once a year (*September*)	Every demo site	GALPAGRO
	(08) Insect vector trap	Every season (4 times a year)		Nutriprado
(IV) Weather	(09) Climatic and atmospheric data	Once a year	Every demo site	AGRODRONE
(V) Quality	(10) Olive Oil (organoleptic characteristics)	Every harvest (1 time a year)	5 strategies / Every demo site	GALPAGRO (SAHC, SALOV)
	(11) *Almond (size and USDA grades)*			
(VI) Water use	(12) Water Use Efficiency (WUE)	Every year after harvest	5 strategies / Every demo site	GALPAGRO (SAHC, SALOV)
	(13) Irrigation Water Productivity (IWP)			
	(14) Stem Water Potential (SWP)	1 time a week: every year from April to October	Replication: Spain (2 strategies)	GALPAGRO
(VII) Carbon Footprint	(15) CO_2 emitted (agricultural processes)	At the end of project	Every demo site	GALPAGRO
(VIII) Biodiversity	(16) Auxiliary fauna (insect populations)	Once a year	Every demo site	GALPAGRO
(IX) Production Value	(17) Money saved	At the end of the Project	Every demo site	GALPAGRO
(X) *Xf* Resilience	(18) Resilient Rate	Spring/Summer 2021 and 2022	Authorized laboratory	UCO (IVALSA)

As instruction on basic monitoring principles, Agrodrone provides a protocol for monitoring and control procedures including sampling methodologies and data gathering (see Annexes, Annex A.2.1).

4. Conclusion(s)

Chapter I on **Introduction to *Xylella fastidiosa*** introduces the pathogen from a theoretical and practical standpoint.

Topics discussed in chapter I include: (i) how is the pathogen spread; (ii) what are the symptoms of related diseases and how to detect its presence; (iii) the subsequent consequences of an infection: monetary and environmental; (iv) distribution of the disease in the EU; (v) containment/elimination regulations; and (vi) the importance of preventing its further spread.

Chapter II about **Best practices to increase resilience** begins with a theoretical lesson and ends with the application of the theory to the farm.

Topics discussed in the second part include: (i) how to decrease water consumption on the farm; (ii) soil and plant health; (iii) an introduction to organic products such as bio-fertilizers and the benefits of using these products over conventional agrochemicals.

The use of auxiliary flora and fauna session in chapter II, details how to choose the appropriate plants and insects according to the region and crop planted, as well as where and how to safely implement the new fauna.

As part of **Monitoring Activities**, after each session, the theories learned will then be demonstrated or shown on a demonstration site, where the trainees can participate in or witness the implementation process before applying it to their own farms.

Annexes

Annex A.2.1

Parameters to be measured by Agrodrone

Satellite image and unmanned aerial vehicles (UAV) present an opportunity to monitor crop fields with high spatial and temporal resolution remote sensing capable of improving management in agriculture. In this project, we applied different remote sensors and compared their performance with ground-truth plant data. Several index will be obtained from satellite image and UAVs to obtain positive correlations related to stress indicators in plants.

I. Tree Temperature

Thermal imagery is also a common remote sensing technology used to assess water stress in plants, via thermal indices (calculated using artificial surfaces as references), estimates of the difference between canopy and air temperature, and even canopy conductance estimates derived from leaf energy balance models.

The thermal flights were provided by the fixed-wing autonomous drone eBee by senseFly. The unmanned aerial system consists of three parts – software for flight planning eMotion v.2, an autonomous fixed-wing drone eBee and a software for post-processing Postflight Terra3D. The last is a production by the company Pix4D. From a photogrammetric point of view, a fixed-wing drone is recommended for mapping arable lands as it provides with autonomous flights to a pre-set flight plan where the lateral and longitudinal overlaps are recommended to be 85%. This value is, of course, higher than the theoretical ones – 80% longitudinal and 60% lateral – as for UAV photogrammetry a bigger overlap is needed in order to general bigger number of keypoints between the overlapping images.

The thermal imagery was provided by the senseFly thermoMAP camera. It is a thermal infrared camera, featuring an integrated shutter for in-flight radiometric calibration. This camera enables capturing video footage and still, images, therefore, enable the creation of thermal maps for water distribution analysis of irrigation lands.

II. Vegetative development (NDVI and NDWI)

Both indices are measured with satellite image and are indicator of the vegetative health of crops.

NDVI (Normalized difference vegetation index)

The NDVI, is an index used to estimate the quantity, quality and development of vegetation based on the measurement (by means of remote sensors commonly installed on a space platform) of the intensity of the radiation of certain bands of the electromagnetic spectrum that vegetation emits or reflects.

The NDVI is linked to a large number of factors in crops. Biomass is usually the most important factor.

NDVI was one of the most successful of many attempts to simply and quickly identify vegetated areas and their "condition," and it remains the most well-known and used index to detect live green plant canopies in multispectral remote sensing data. Once the feasibility to detect vegetation had been demonstrated, users tended to also use the NDVI to quantify the photosynthetic capacity of plant canopies.

Live green plants absorb solar radiation in the photosynthetically active radiation (PAR) spectral region, which they use as a source of energy in the process of photosynthesis. Leaf cells have also evolved to re-emit solar radiation in the near-infrared spectral region (which carries approximately half of the total incoming solar energy), because the photon energy at wavelengths longer than about 700 nanometers is too large to synthesize organic molecules. A strong absorption at these wavelengths would only result in overheating the plant and possibly damaging the tissues. Hence, live green plants appear relatively dark in the PAR and relatively bright in the near-infrared. By contrast, clouds and snow tend to be rather bright in the red (as well as other visible wavelengths) and quite dark in the near-infrared. The pigment in plant leaves, chlorophyll, strongly absorbs visible light (from 0.4 to 0.7 µm) for use in photosynthesis. The cell structure of the leaves, on the other hand, strongly reflects near-infrared light (from 0.7 to 1.1 µm). The more leaves a plant has, the more these wavelengths of light are affected, respectively. Since early instruments of Earth Observation, such as NASA's ERTS and NOAA's AVHRR, acquired data in visible and near-infrared, it was natural to exploit the

strong differences in plant reflectance to determine their spatial distribution in these satellite images.

The NDVI is calculated from these individual measurements as follows:

$$\mathrm{NDVI} = \frac{\rho_{\mathrm{NIR}} - \rho_{\mathrm{Red}}}{\rho_{\mathrm{NIR}} + \rho_{\mathrm{Red}}}$$

Where red and NIR stand for the spectral reflectance measurements acquired in the red (visible) and near-infrared regions, respectively. These spectral reflectances are themselves ratios of the reflected over the incoming radiation in each spectral band individually, hence they take on values between 0.0 and 1.0. By design, the NDVI itself thus varies between -1.0 and +1.0. It should be noted that NDVI is functionally, but not linearly, equivalent to the simple infrared/red ratio (NIR/VIS). The advantage of NDVI over a simple infrared/red ratio is therefore generally limited to any possible linearity of its functional relationship with vegetation properties (e.g. biomass). The simple ratio (unlike NDVI) is always positive, which may have practical advantages, but it also has a mathematically infinite range (0 to infinity), which can be a practical disadvantage as compared to NDVI. Also, in this regard, note that the VIS term in the numerator of NDVI only scales the result, thereby creating negative values. NDVI is functionally and linearly equivalent to the ratio NIR / (NIR+VIS), which ranges from 0 to 1 and is thus never negative nor limitless in range.[7] But the most important concept in the understanding of the NDVI algebraic formula is that, despite its name, it is a transformation of a spectral ratio (NIR/VIS), and it has no functional relationship to a spectral difference (NIR-VIS).

NDWI (Normalized difference water index)

NDWI was proposed to assess water status by the combination of NIR and short-wave infrared (SWIR) channel because both are in the high reflectance plateau of vegetation canopies and sense similar depth in vegetation canopies. Absorption by vegetation liquid water near NIR is negligible, and weak liquid absorption near SWIR is present. Therefore, canopy scattering enhances the water performance.

$$\mathrm{NDVI} = \frac{\rho_{\mathrm{NIR}} - \rho_{\mathrm{Swir}}}{\rho_{\mathrm{NIR}} + \rho_{\mathrm{Swir}}}$$

This index serves to evaluate the degree of humidity of the crop mass, in this way we can evaluate the actions concerning irrigation on the surfaces of the project, compare the evolution of them, and try to detect these forced deficiencies through deficit treatments in a remote way.

The information necessary to develop the indexes will be obtained through the satellite Sentinel 2, belonging to the European space agency. The satellite provides free of charge the information generated by its sensors. In each of the moments proposed in the project, the satellite information will be downloaded through an API that automates said process. This information will be used to generate the previously described indexes, which will be projected through a GIS software for later use in the analytical results obtained during the project.

III. Available Water Capacity (AWC)

The spatial variability of soils is one of the main problems faced when planning irrigation management, especially when large tracts of agricultural land are involved. Parameters such as soil texture or soil water content are fundamental for understanding the determining factors of a soil with respect to water. Available water capacity (AWC) is a vital indicator when considering soil properties from the point of view of irrigation management. A geostatistical methodology will be used to develop efficient predictive maps for soil characterization from the point of view of irrigation with the help of guided soil sampling based on the Apparent Electric Conductivity (ECa). Ordinary and regression kriging models will be used to generate predictive maps of AWC.

The ECa survey will be conducted with a soil electrical conductivity sensor. Sensor generates two sets of data: topsoil data comprising shallow soil ECa from 0 to 30 cm and deep soil from 0 to 90 cm. An ARVATEC monitor with a Topcon HiPer Pro-GPS (Topcon Corporation, Tokyo, Japan) and Maxor-GGDT (Javad Navigation System, San José, CA, USA) base with sub-meter accuracy will be used to georeference the ECa measurements. Latitude and longitude and shallow and deep ECa data will be recorded at 1 s intervals on the sensor data logger in an ASCII text format. Later, this raw ASCII file will be transferred to other software for further analysis. ECa measurements will be made along different parallel transects approximately 20 m apart and 4 m between each

measurement. Ordinary kriging will be used to develop an ECa map for the plots. These maps will be used for guided soil sampling of soil properties, taking into consideration a good sample distribution over the different plot surfaces and trying to cover the different ECa ranges. Soil samples will be taked covering homogeneous zones described by the ECa prediction maps. The soil samples will be placed in plastic bags, air-dried and analysed for particle-size distribution by gravitational sedimentation using the Robinson pipette method (Soil Conservation Service, 1972) after passing the fine components through a 2 mm sieve. Calculation of AWC was made using the following equation:

AWC = field capacity (FC) – permanent wilting point (PWP)

The equations of Saxton *et al*. (1986) will be used to calculate FC and the PWP values of each type of soil.

Annex A.2.2

Soil enzimatic activity determinations

I. Dehydrogenase, DHA

Methods and techniques

Analysis of the activity DHA by the method of Trevors *et al*. (1982) modified by Garcia *et al*. (1993).

Quantitation of INTF compound formed in soil incubated with INT. Used INT as an artificial electron acceptor as a substrate to determined DHA in soil.

Protocol

- Processing samples: use wet soil and standard control.
- Determination soil water content to 60% of soil field capacity.
- Add INT 0.4% solution to samples and incubate 20 hours in darkness.
- After incubation time, add 10 ml methanol to all samples.
- Stop the reaction shaking and filtering the content of the samples.
- Construct a calibration curve with a solution of known concentration of INTF.
- Spectrophotometric reading of the results.
- The results are expressed in µmol INTF $g^{-1}.h^{-1}$.

II. Urease

Methods and techniques

Sinsanbaugh *et al*. (2000).

Determination of ammonium released in the hydrolysis of urea by colorimetry.

Protocol

- Processing samples: use wet soil and standard control.
- Prepare a buffered soil solution.

- Add urea solution to samples.
- Incubate 18 hours.
- After incubation time, add salicylate and cyanide solution to all samples.
- Incubate 20 minutes.
- Construct a calibration curve with a solution of known concentration of ammonium solution.
- Read the results with a Spectrophotometer.
- The results are expressed in µmol $N\text{-}NH_4^+$ $g^{-1}.h^{-1}$.

III. Catalase

Methods and techniques

Johnson and Tempe (1964)

Determination of H_2O and O_2 with H_2O_2 using permangatometry valoration.

Protocol

- Processing samples: using wet soil, blank and control samples.
- Valuation of samples per permangatometry.
- Realization of calculations.
- The results are expressed in mmloes H_2O_2 g^{-1} h^{-1}.

IV. Phosphatase

Methods and techniques

Determination of phosphodiesterase activity according to Bowman and Tabatabai Bremner (1978).

The method is based in spectrophotometric determination using the p-nitrophenol released from the soil. Soil is incubated at 37°C for 1 hour in a buffered solution (pH 8.0) of bis-p-nitrophenyl phosphate.

Protocol

- Processing soil samples.
- Preparation of standards blanks and samples.
- Spectrophotometric reading of the results.
- Realization of calibration curve.
- Results determination using mathematical formula.
- The results are expressed in µmoles *p*-nitrofenol liberado g^{-1} h^{-1}.

V. β-Glucosidase

Methods and techniques

Tabatabai (1982).

Colorimetric determination of p-nitrophenol after the action of β-glucosidase and after soil incubation with a substrate in buffered medium.

Protocol

- Processing samples: using wet soil and standard control.
- For enzymatic activity measurement, control and standards are incubated. The incubation of samples is realized with the substrate solution.
- Standard and control are incubated without substrate but with increasing concentrations of p-nitrophenol.
- After incubation, measure absorbance and do mathematical calculation.
- The results are expressed in µmol PNF g^{-}1dry soil h^{-1}.

Annex A.2.3

Diagnostic protocol adopted by the Standards Committee on behalf of the Commission on Phytosanitary Measures in August 2018. The annex is a prescriptive part of ISPM 27.

ISPM 27, Diagnostic protocols for regulated pests; DP 25: *Xylella fastidiosa*. Adopted 2018; published 2018.

LIFE RESILIENCE