



Integrated Pest Management protocol against oak decline in Mediterranean ecosystems

Part of deliverable D4.1 of workpackage 4
Large-scale application new customized IPM protocols

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Project acronym: LIFE FAGESOS
Project title: Phytophthora-induced decline of Fagaceae ecosystems in Southern Europe exacerbated by climate change: preserving ecosystem services through improved integrated pest management

Grant Agreement number: 101074466
Call identifier: LIFE-2021-SAP-CLIMA
Start date of the project: 01/09/2022
Duration: 60 months
Website: www.lifefagesos.it & [EU Portal](#)



This document has been produced with financial support from the European Union's LIFE Programme under grant agreement No 101074466. Views and opinions expressed are those of the author(s) only and do not necessarily reflect those of the European Union or CINEA. Neither the European Union nor CINEA can be held responsible for them.



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1. Executive Summary

Forest decline is a major challenge for ecosystem management. Alien invasive forest pathogens (AIFPs) and their effects, exacerbated by climate change, pose a major threat to the socioeconomic and ecological sustainability of Mediterranean oak and chestnut species. One of the main objectives of the LIFE FAGESOS project is to develop tailored Integrated Pest Management (IPM) protocols, based on ecologically friendly products and management recommendations.

During the project's first phase, different products were tested to mitigate the impact caused by *Phytophthora cinnamomi* on *Quercus suber* (cork oak) and *Q. ilex* (holm oak) plants. This document provides the first release of a detailed IPM protocol for evergreen oak (holm and cork oak) “dehesas” and stands based on the test results. It includes contextual information about the problem and the treatments, detailed information about selected products and their application, recommendations for the application of hygiene and control measures, and general outlines for the management of degraded sites.

2. Introduction

Integrated pest management (IPM) is a pest control strategy based on environmentally sustainable solutions aiming to prevent a long-term basis the impact of pests and diseases by combining monitoring plans, early detection, use of biocontrol agents Plant Growth Promoting (PGP) microorganisms, habitat manipulation, and modification of cultural practices among other tools. Treatments, preferably based on environmentally safe molecules, are used only after the monitoring indicates they are needed, according to established guidelines. Moreover, pest control products should be selected and applied in a way that minimizes risks to human health, beneficial and nontarget organisms, and the environment.

Rather than directly targeting the pest, the IPM strategy aims to create environmental conditions unfavorable to the pest agents, while reinforcing the ability of the host plant/population to react by increasing their resilience.

IPM programs combine management approaches for greater effectiveness. The most effective, long-term way to manage pests is by using a combination of methods that work better together than separately. Approaches for managing pests are often grouped into the following categories.

I) Biological control

Biological control is the use of natural enemies—predators, parasites, antagonists, and competitors — to control pathogens and their damage. A broader concept also includes molecules produced by natural enemies.

II) Cultural controls

Cultural controls are practices that reduce pest establishment, reproduction, dispersal, and survival. For example, applying hygienic measures to machinery, vehicles, and staff to reduce the dispersal of the pathogen, reduce the intensity of exploitation, or applying soil fertilization treatment to increase the vitality of the plant.

III) Mechanical and physical controls

Mechanical and physical treatments aim to control the pest by directly killing, blocking, or making the environment unsuitable for the pathogen. Traps for rodents are examples of mechanical control. Physical controls include mulches for weed management, steam sterilization of the soil for disease management, or barriers such as screens to keep birds or insects out.

IV) Chemical control

Chemical control is the use of pesticides. In IPM, pesticides are used only when needed and in combination with other approaches for more effective, long-term control. Pesticides are selected and applied in a way that minimizes their possible harm to people, nontarget organisms, and the environment. In IPM strategies, the choice of selective molecules is encouraged to minimize the impact to non-target organisms and to preserve air, soil, and water quality.

3. Mediterranean evergreen oak decline

Oak decline is one of the major challenges that Mediterranean ecosystems are facing in the last decades (Jung et al., 2000; Burgess et al., 2017; Ruiz-Gómez et al., 2019). Holm oak and cork oak are the most threatened species due to the mortality episodes of the oak decline, mostly driven by the root rot caused by the alien invasive forest pathogen (AIFP) *Phytophthora cinnamomi*.

Cork oak (*Quercus suber* L.) ecosystems are of great relevance in terms of economic, cultural, and ecological value in the Mediterranean regions (Aronson et al., 2009). Over the last three decades, an increased decline of cork oak trees has been reported as a growing problem across its geographic distribution (Brasier et al. 1993; Camilo-Alves et al. 2013; Moricca et al. 2016). Several factors, including diseases and pests, wildfires, climate change, overgrazing, degradation, and fragmentation have been associated with cork oak decline (Jung et al., 1996, Sanchez et al., 2002, Gomez-Aparicio et al., 2012). However, the soilborne pathogen *Phytophthora cinnamomi* is one of the main drivers of this phenomenon (Brasier et al., 1993, Robin et al. 1998; Scanu et al. 2013), although many other *Phytophthora* species are also involved (Seddaiu et al. 2020; Aurangzeb et al. 2023) Symptoms of cork oak decline are crown thinning, resulting in a significant increase of crown transparency; the presence of epicormic shoots on twig and branches; at the root level, progressive necrosis of fine feeder roots and the presence of cankers on the more large and suberized roots; bark necrosis, associated with blackish exudate, which occasionally bounds the whole stem (Brasier, 1996; Robin et al., 1998; Scanu et al., 2013) (Fig. 1).

Holm oak (*Quercus ilex* L.) is one of the more extended species over the Mediterranean basin, and the main species dominating the tree layer of “dehesas” ecosystem covering an estimated surface over 4.6 mill ha in the Iberian Peninsula. The figures of holm oak distribution in this area account for around 2.2 mill ha of “dehesas” rangeland ecosystems of *Q. ilex* (Díaz Esteban and Pulido Díaz, 2009), besides another 1.42 mill ha of holm oak natural Mediterranean forests (Rodá et al., 2009).

The holm oak decline, also known as “La Seca” syndrome, is devastating entire areas of the western part of the Andalusian region (Andévalo) and the Sierra Morena mountains, due to the death

of young and mature trees (Ruiz-Gómez et al., 2019) and the failure of afforestation (Sánchez-Cuesta et al., 2022). It is considered that the main factor triggering tree mortality is the root rot caused by the Alien Invasive Forest Pathogen (AIFP) *Phytophthora cinnamomi* and other *Phytophthora* species.



Figure 1. Severe dieback and mortality of cork oak trees due to *Phytophthora* infection

The symptoms of root rot are nonspecific and become identifiable when most of the root system is already compromised, except in the case of sudden death, in which case the problem is only identifiable when the tree is dead (Carrasco Gotarredona et al., 2009) (Figure 2)

Although there is no accurate data on the incidence or the extent of holm oak decline, in the year 2010, official sources estimated the loss of holm oak stands at more than 8000 ha per year only in Spain (Senado, 2010). This decline causes severe environmental and economic losses in a particularly vulnerable ecosystem located in rural areas, exacerbated by other problems such as intensification of agricultural practices and livestock farming, land withdrawal and abandonment, and the direct effects of climatic change (Hernández-Lambraño et al., 2018; Sánchez-Cuesta et al., 2021). Thus, the protection of the “dehesas” ecosystem and the recovery of already degraded areas is at the front line of the forest management policies of the regional administration.

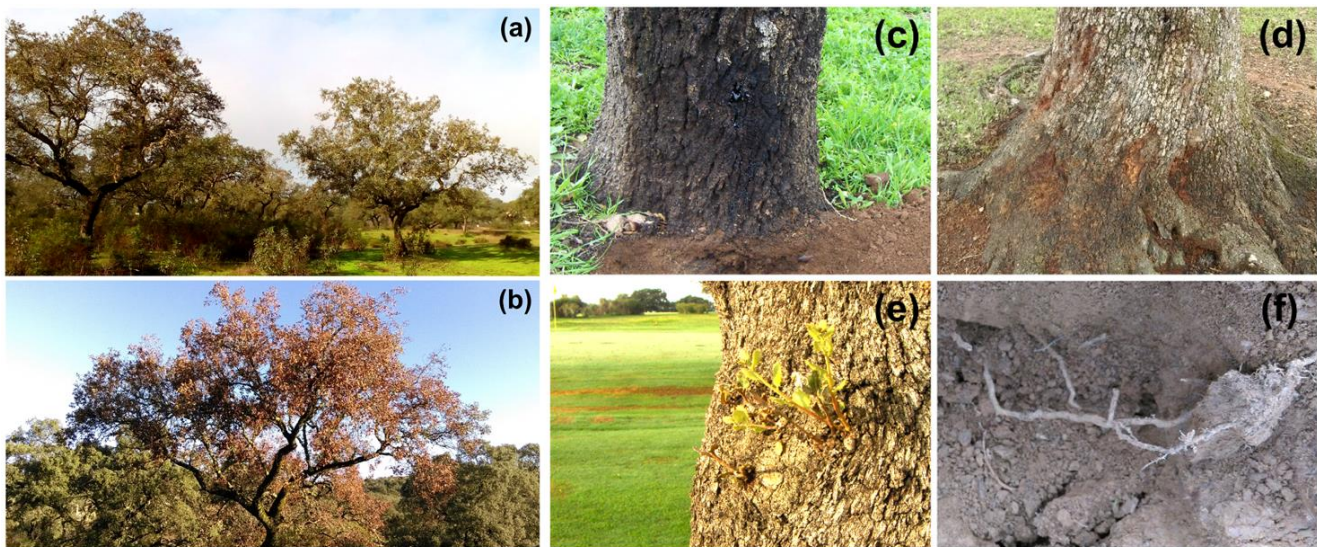


Figure 2. Holm oak main decline symptoms. (a) regressive decline with progressive defoliation. (b) sudden death. Blighted foliage remains on the tree crown. (c) canker at the base of the trunk. (d) galleries of wood-drilling insects with sawdust at the base of the trunk. (e) epicormic sprouts on the trunk. (f) fine root loss on superficial absorbent roots. Source: Author's archive.

Epidemiology of the disease is associated with climatic conditions and management, along with other relevant factors such as soil quality and pollution. Average precipitation is not a main factor, as *Q. ilex* is a sclerophyllous species well adapted to dry areas, but heavy rainfall episodes and soil flooding are related to mortality and heavy defoliation episodes. Moreover, the high recurrence of extreme drought events and the global rise of temperatures seems to exacerbate these episodes, probably due to their effects on the global health status of the holm oak stands (inciting factor) (Sánchez-Cuesta et al., 2021). Extended periods of drought, waterlogging, unpredictable fluctuations in water levels, sandy or shallow soils, unexpected heavy rainfall, as well as opportunistic pathogens and pests, can accelerate the disease progress and lead to sudden wilting and death of the trees (Brasier et al., 1993; Jung et al. 1996; Balci & Halmschlager 2003; Jönsson et al., 2005; Moreira & Martins, 2005). Agreeing with the patterns seen in chestnut, the association of ink disease with natural drainage lanes and the network of forest and local roads was demonstrated (Cardillo et al., 2018).

Integrated management of holm oak decline is difficult and must take into consideration several variables and factors associated with its spread, incidence, and severity. Such variables can be considered within a general context of area-wide and landscape heterogeneity. Physical and human infrastructures such as the existing network of natural drainage and roads offer preferential corridors of the spread and dispersal of *Phytophthora*. Other variables include cultural practices and host resistance. Also relevant is the pressure exerted by activities related to specific land uses, such as the presence of nurseries or another type of plantation in the neighboring areas that could act as a bridge for host shift and the introduction of pathogens in disease-free areas. Last, but not the least, the quality and level of monitoring of the territory could alter the application efficacy of control measures (Vannini and Morales-Rodríguez, 2019).

4. Sustainable products to be applied in IPM

Instead of an instinctive reaction to a specific problem, “holistic” pest management is a series of interrelating processes that are incorporated into the entire spectrum of IPM strategy. Holism is the theory where systems, and each part of a system, should be viewed as a whole and not as isolated parts. “Holistic,” then, is an approach that looks at the big picture and considers all parts. Holistic pest management is an integrated and preventative approach that considers the overall health of the plant and the tree environment to prevent problems and manage them wisely if they arise.

The strategy of this IPM is based on applying the correct hygiene measures described in section 5 to avoid the spread of the pathogen and the three-step treatment of the affected areas. These treatments are based on: 1) the application of a fungicide to lower the concentration of the inoculum present in the soil; 2) the incorporation of microorganisms that occupy the space left by the pathogen, with the antagonist function against the pathogen and stimulation of plant growth; 3) treatments with plant defense inducers that protect the tree from the impact of the pathogen (Figure 3).

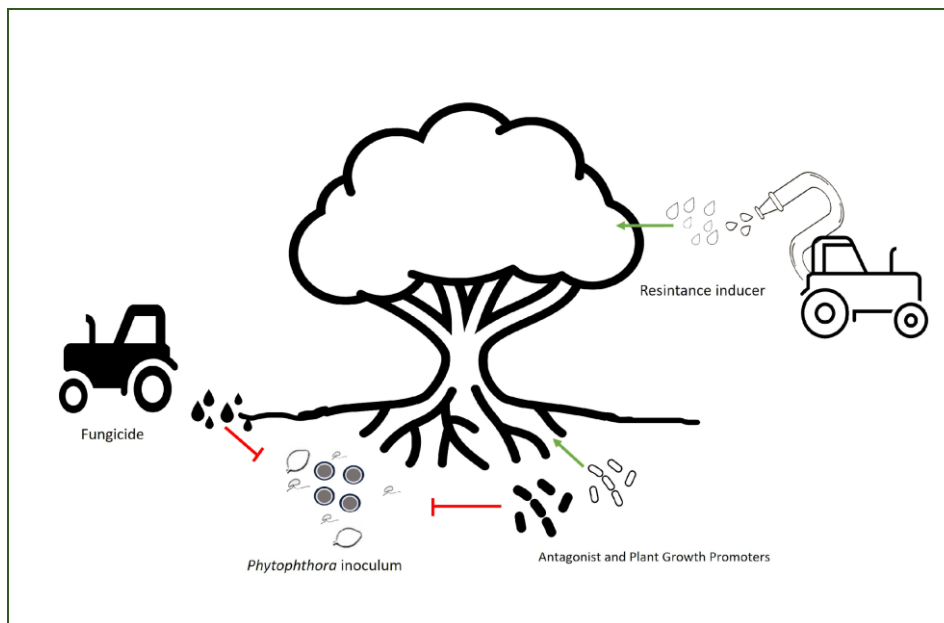


Figure 3. Holistic approach of treatment to mitigate the impact of *P. cinnamomi* in chestnut orchards

4.1 Product formulation and dose

4.1.1 Reduction and control of oomycete inoculum levels

Brassica plants contain high concentrations of glucosinolates (GLS) in specific cell types that are part of the natural defense systems of these plants (Koroleva et al. 2010). These molecules are a substrate for hydrolytic enzymes (myrosinases), stored in vacuoles or specific myrosin cells of Brassicaceae plants. Following tissue disruption, GSLs come into contact with myrosinases and are

hydrolysed to isothiocyanates (ITCs). The ITCs are known to have broad biocontrol activity against arthropods, fungi, and oomycetes (Brown and Morra 1997; Rosa et al., 1997).

The product chosen in the FAGESOS mesocosm tests to control the inoculum levels of *P. cinnamomi* in the soil is a commercial product, already available for its use as fertilizer (BioFence FL, Nutrien Italia S.p.a., Livorno, IT). In its commercial form, BioFence is produced from *Brassica carinata* selection ISCI7 using a partial defatting method that limits GSL and myrosinase degradation, in this way optimizing product efficacy (Lazzeri et al. 2004). The biocontrol efficacy (96,5%) of Brassica pellets on *P. cinnamomi* has been reported in vitro and mesocosm experiments providing an alternative to synthetic pesticides within disease management programs (Morales-Rodriguez et al., 2016). In addition to its biocidal activity, this product is reported to have a positive and durable effect as a soil fertilizer. It furthermore favors the emission of new roots, improving plants' capacity to absorb nutrients and therewith resilience against abiotic stress (e.g. extreme temperatures and drought).

BioFence is commercially available as a pellet for dry applications and as a two-component product (Figure 4), consisting of a liquid formulary and brassica flour to be combined and applied as a soil drench. Both formulations have been compared for efficacy in mesocosm trials being the liquid formulation the one with the best performance reducing *P. cinnamomi* inoculum.

Composition: Biofence FL is a two-component product, including liquid formulation and flour derived from pellet of selected *Brassicae* species. The pack includes a filter bag to ease the infusion process.

Nutritional contribution:

- Organic nitrogen powder 6%
- Total sulphur 15%
- Magnesium 0.5%
- Free amino acid content in total N >50%

-Liquid organic nitrogen 3%



Figure 4. Biofence FL. 10 liters of formulation + 5 KG of flour

Preparation:

1) Shake the liquid formula to mix the components well and dilute it in a few liters of water using a large container (Figure 5A).

Approx. 4 L of water for 1 L of liquid formulation.

2) Use the powder necessary for the treatment, pour it into the special filter, and then immerse the filter in the solution prepared before (Figure 5B).

0.9 kg of flour for 1 L of liquid formulation and 4 L of water.

For the whole commercial pack (5 Kg of pellet flour and 10 L of liquid formulation), use 22 L of water. Prepare it in a container of 50 L of volume

3) Leave the flour to infuse for at least one hour but not more than 4/5 hours. During this time, shake the filter regularly so that all the flour comes in contact with the liquid solution.

4) After the minimum infusion time is over, lift the filter from the liquid compound and keep it suspended to be able to squeeze it and therefore allow the isothiocyanate to come out and be integrated into the solution.

5) Rinse the filter in another container with clean water. Shake the filter to allow the maximum amount of product to be dissolved in water. Then, squeeze the filter and incorporate the water into the infusion.

For the whole commercial pack, rinse in a volume of 10 L.

6) The solution obtained must be brought to the volume necessary in the fertilizer tank, to be able to distribute it in the soil around the tree, making sure to spray especially the base of the plants under the crown projection area.

For the whole commercial pack, at this point, we will have about 40-41 L of infusion. Mix with 1460 L of water to have a final volume of 1500 L.



Figure 5. Preparation of Biofence FL. A) Mix the liquid solution in 22 L of clean water and prepare the pellet flour introducing it in the filter bag. B) Immerse the filter bag in the mixture of water and liquid solution. Shake vigorously to improve infusion. C) After 1 h, rinse the filter bag and add the solution in a fumigation or fertilizer tank with 1460 L of water.

Mode of application and dosage: (for 1 pack, 1500 L of final dilution; 35-45 trees)

- The product should be applied only on trees affected by illness and/or located in the front of disease foci advance.
- The product should be applied by treating the surface below the crown of the tree in early spring after a rainy period. The objective is to kill the inoculum in the outer layers of the soil (10-15 cm) to reduce the risk of dispersal through the passive movement of contaminated soil particles.
- Apply the adequate volume of solution depending on the tree size (Table 1):

Table 1: solution volume for soil application depending on crown size.

Crown diameter (m)	Solution volume (L)
< 10	25
10 to 20	35
> 20	45



ATTENTION!!! USE THE SOLUTION WITHIN 4 HOURS OF PREPARATION

4.1.2 Improving tree health through soil microbiome.

This treatment is aimed at improving the plant physiology and to limit the habitat suitability for the pathogen through amendment with bioproducts based on plant growth-promoting microorganisms and antagonist *Trichoderma* spp. Soil bio-amendment with plant growth-promoting microbes (PGPM) is an environmentally friendly alternative to chemical fertilization. These organisms can colonize plant roots, providing benefits to their hosts: they increase the availability of soil nutrients, and the resistance against pathogens, by modulating the production of phytohormones. So, they mitigate biotic and abiotic stresses and increase plant production.

Trichoderma is a genus with high potential in biological control and IPM implementation. *Trichoderma* comprises several opportunistic, avirulent plant symbionts, being major colonizers of soils in all types of ecosystems, able to control many soil pathogens through diverse mechanisms such as plant resistance induction, direct competition, inhibition, and parasitism (Vinale et al., 2008). Opportunistic plant symbionts are organisms that are not obligated to be symbionts to survive, but they thrive considerably more when having a host plant. Some *Trichoderma* strains are well known as proficient biocontrol agents, decreasing the severity of plant diseases, primarily in the soil or on plant roots (Sharma and Sharma 2020). These effects have been successfully confirmed in research for *Phytophthora* spp., controlling or even reducing *Phytophthora*-related diseases (Aleandri et al., 2015; Sharma and Sharma 2020, Ruiz-Gómez and Miguel-Rojas, 2021).

Among the products tested in the FAGESOS mesocosm trials, all the products based in PGPB and 2 products based on *Trichoderma* spp. had a positive effect on the health status of infected oak plants. Eventually, two products were chosen for the IPM protocols due to their effectiveness, and also considering their current availability in the market: Bactrium® and Tricoten® of Atens (Agrotecnologías Naturales, S.L., Tarragona, Spain).

Bactrium® (Fig. 6) has highly competitive rhizosphere bacteria that colonize the root system. These bacteria improve plant nutrition, improve the efficiency of fertilizer use, and unblock elements blocked in the soil. Both *Bacillus megaterium* strains contained in Bactrium have been fully sequenced at NGA lab. Thanks to this work we can affirm that they contain coding genes that contribute to:

- The uptake and assimilation of nitrogen (Nitrite Transporter and enzymes of the Nitroreductase family)
- Increased resistance to copper contamination (Copper Resistance Protein)
- Iron uptake (siderophores)
- Phosphorus solubilization (acid phosphatases and phytases)

Composition:

- *Bacillus megaterium* MHBM06: 5x10⁹ UFC/g
- *Bacillus megaterium* MHBM77: 5x10⁹ UFC/g
- Total rhizosphere bacteria: 1x10¹⁰ UFC/g



Figure 6. Bactrium®, Atens

Tricoten® is a wettable powder formulation based on *Trichoderma atroviride*. Its effectivity controlling pathogens such as *Fusarium*, *Sclerotinia* and *Botrytis* has been already proven, and it has been demonstrated to reduce significantly the a significant reduction on the disease symptoms in oaks infected with *Phytophthora cinnamomi* in the mesocosm trials carried out in the FAGESOS project. There are other formulations with *T. atroviride* available in the market, although we cannot provide information about their effectivity in holm or cork oak against *P. cinnamomi* because they were not included in the FAGESOS mesocosm trials. However, if their formulation is based in *T. atroviride*, it would be assumed that the effects might be similar will be similar. For Italy, Tricoten® is not yet available, although at the moment of writing of this document, it is in the process of registration.



ATTENTION!!! DO NOT USE PRODUCTS BASED ON *T. KONINGII*.

This microorganism can stimulate homothallism in P. cinnamomi

(Pratt et al., 1972; Brassier, 1978)

Composition:

- *Trichoderma atroviride* AT10, conidial wettable powder, 5x10⁹ UFC/g



Figure 7. Tricoten®, Atens®

Mode of application and dosage:

- Dosage of Bactrium: 2L / ha
- Dosage of Tricoten: 1 Kg / ha
- Both products can be applied directly to the soil, through spray bar, dissolved in the necessary water, depending on the consumption rate of each spray bar.

Example: For a tank of 2000 L with spray bar of 6 m, at a rate of 200 L /ha, add 20 L of Bactrium and 10 kg of Tricoten for 10 ha.

- The treatment will be applied together in the spring during the months of March-April, preferably after a rainy period.

4.1.3 Resistance inducer

Plant resistance inducers (PRIs) could be useful to reduce the use of pesticides. They are molecules of different nature that lead to improved protection from pathogen attacks by inducing the plant's defense mechanisms, so-called Plant Triggered Immunity (PTI). They are also referred to as plant resistance or defense effectors. PRIs are known to be effective against various pathogens, including viruses, bacteria, oomycetes, and fungi attacking plants. This treatment is highly recommended in the oak decline because *P. cinnamomi* is not a primary pathogen of holm and cork oak but a triggering factor of mortality in the decline symptom. Thus, improving the natural defense mechanisms of trees will protect them against threshold events which can lead to heavy root rot episodes and tree death.

Among the products tested in the FAGESOS mesocosm trials, the product presenting the highest effectiveness in reducing symptoms and mortality in the infected plants was Kalex EVO® (Alba Milagro International S.p.A.; Parabiagio, Italy). Kalex EVO® is a natural defense booster including fertilizer substances (copper and molybdenum) and several organic activator compounds (carboxylic acid, polysaccharides, and natural extracts), proven as effective against a wide range of fungal and bacterial diseases. KALEX EVO is formulated so that the substances it contains are rapidly absorbed and transported from the roots to the leaves and vice versa. Due to its carrier effect, it can be combined

with pesticides, to improve their system and functioning, after checking compatibility. Do not use it during blooming.

Composition:

- Cooper (Cu), 4.1 % w/w
- Molybdenum (Mo), 0.03 % w/w
- Vegetal extracts: Polysaccharyde, vitamins, minerals, fats, amynoacids, olyphenoids and pigments.
- Cartboxylic acid



Figure 8. Kalex EVO® commercial presentation, Milagro International S.p.A

Mode of application and dosage:

- The recommended treatment consists of 2 foliar applications with an interval of 15 days between them.
- Application must be done in spring (April-May), after blooming, and without foreseen rain in the following 24 hours.
- Dosage: 2 L /ha.
 - In the case of a common oak “dehesa” (both holm and cork oak) with a tree density ranging between 60 and 90 trees /ha, a 2000 L tank can be prepared adding 20 L of product and applying it following the recommendations of Table 2.
 - To control oak decline in young afforestation or dense plantations, use a dose of 3 L/ha

Table 2: Orientative solution volume for crown spray application in holm oak depending on crown size

Crown diameter (m)	Solution volume (L)
< 10	18
10 to 20	25
> 20	32

4.2 Period of application and timeline

The treatments should follow a determined order, starting by inoculum reduction, as a critical step. This product should be applied after a rainy period, over wet soil, to ensure penetration, and coincide with the maximum probability of *P. cinnamomi* proliferation activity period.

After the treatment with BioFence FL, a period of 30 days should be observed before treatment with microorganisms or any other bioproduct, to ensure that isothiocyanates are degraded, to avoid interference with the amendment.

Regarding foliar treatment, the most important aspect to consider is the blooming. The product must be applied when the flowering period is over and the flowers have already been fertilized to ensure better fruit production.

Observing all these aspects, and taking as an example the calendar of the Demonstrative area of La Tejera, the proposed calendar for application in “dehesas” would be as follows (Table 3):

Table 3: Treatments calendar for the FAGESOS Demonstrative Area of La Tejera (Villaviciosa de Córdoba, Spain)

	Week					
	I	II	III	IV	V	VI
BioFence	20-21 March					
Kalex EVO				18-19 Apr		1-2 May
Bactrium+Tricoten				22-23 Apr		

Dates should be adjusted taking into account rainy episodes and flowering periods.

In the case of plantations or natural evergreen forests not linked to Iberian pig farming, it is possible to advance the Biofence treatment by carrying it out during the winter when the soil is humid and grass growth is less. An example of treatment in these areas is shown in Table 4.

Table 4: Treatments calendar for plantations or natural evergreen forests

	Week					
	I	II	III	IV	V	VI
BioFence	20-21 Feb					
Kalex EVO				18-19 Apr		1-2 May
Bactrium+Tricoten		22-23 Mar				

Dates should be adjusted taking into account possible rainy episodes and flowering periods.

5. Hygiene measures

Hygiene measures are preventive methods to avoid passive transport of *P. cinnamomi* inoculum from the infection foci to healthy areas. Anything favoring the passive transport of infected soil can act as a carrier of the disease. This includes vehicle tires, footwear, and wild and farm animals. Vannini et al. (2021) demonstrated that most of the inoculum moves along the network of roads bordering or crossing chestnut orchards and the network of superficial water drainages, agreeing with other works in holm oak “dehesas” (Cardillo et al., 2018). Thus, hygiene measures are a pivotal part of IPM strategy.

Vannini et al. (2010) suggested the following hygiene precautions and practices:

1. The restriction of the transit of people, animals, and machinery in infected areas (foci) and along roads and trails crossing or bordering the affected groves and /or orchards. This measure is very relevant during heavy rain episodes and flooding periods.
2. The disinfection of tires and shoes when exiting from infected areas, particularly in the wet season.
3. The management of superficial water flows by collecting and channelling runoff water from affected areas.

Measures as listed in points (1) and (2) are cost-effective, but their successful application depends on education and awareness among the chestnut stakeholders and private citizens. The latter measure is costly and not easy to implement in large infection foci.

In the case of hygiene measures, everyone's support is needed; a multi-stakeholder approach is highly recommended, where it is implemented in close collaboration with local municipalities, non-profit associations, citizen groups, associations of forest owners and growers, and other interested parties.

Within the FAGESOS project, the following hygiene measures will be implemented and maintained in the demonstrative areas in Spain and Italy:

l) Track stabilization and ditch improvement: Farms and forest areas are full of country roads used for cultural and livestock activities. Most of the time, the road surfacing is highly unstable, prone to mud puddles and potholes able to contain the pathogen inoculum, which can be spread by cars and heavy machinery. In this case, as most of the roads consist of natural soil compacted platforms, with little soil movement, it is easy and cost-effective to repair the road surface with a simple beam or smoothing machine. Moreover, ditches along the main roads crossing the affected areas will be cleaned and improved to collect the water flood, channeling runoff to the main water courses, and thus limiting inoculum spread (Fig. 9)



Figure 9: Country road improvement in La Tejera, Road surfacing can be easily compacted with a heavy beam. Ditches should be cleaned in the runoff areas near foci and/or heavily affected areas, channelling runoff to the main water courses.

II) Footwear cleaning stations: These stations consist of a brushing module to remove soil. The footwear cleaning stations of the FAGESOS project also included lateral devices to remove the mud excess before cleaning shoes, lateral brushes to help with the cleaning, and a spraying module with disinfectant integrated into the brushing module. (Figure 10).



Figure 10: Footwear cleaning stations designed for the FAGESOS project. The stations included lateral brushes, lateral plates for removing excessive mud, and a disinfectant dispenser.

III) Vehicle cleaning stations are an important preventive hygiene measure. In the case of country roads consisting of natural soil compacter platforms, the most important aspect to be considered is the cleaning of tires and wheel arches with disinfectant. The most cost and time-effective measure for this

purpose is the installation of tire baths in the main exit points of the country roads of the farm/estate (Fig. 11). The tire cleaning baths should be filled with a disinfectant solution and must be deep enough to efficiently wet the entire wheel arch at low speed, without posing a significant obstacle for the vehicle.



Figure 11: Example of tire bath at the main entrance of a farm.

The footwear cleaning stations and the tire baths must be provided with a disinfectant solution that does not pose a danger of environmental contamination, to the soil, vegetation, and fauna. For this purpose, quaternary salts-based disinfectants offer an optimal solution. These disinfectants, in an adequate dose, do not harm animals and are harmless to vegetation. In the framework of the FAGESOS project, several products based on these substances and without chemical or contaminant substances in their composition, have been tested in the laboratory. Among the tested products, and also considering their composition, price, and availability in Spain, the product chosen for inclusion in the demonstrative actions of FAGESOS was Sanibacter® (Quimpa S.L.; Madrid, Spain) (Fig. 12). Among the tested products, and taking into account their composition, price, and availability in Italy, the product chosen to include in the demonstrative actions of FAGESOS is Blu (Arco chemical group), the product is used by diluting it in water in a proportion of 1/200. In such a manner, a 1l package will produce a total of 200 liters of disinfectant liquid.

Composition:

- Didecyldimethylammonium chloride (4.5% v/v)
- Excipients



Figure 12. Sanibacter®, Quimpa S.L

Tests included different doses, to find the minimum dose able to effectively kill *P. cinnamomi* inoculum (Table 5).

Table 5: Dosage of Sanibacter® for different applications. The dose indicates the concentration at which no viable inoculum of *P. cinnamomi* was recovered from the tests.

Application	Dose	Recommendation
<i>Laboratory tests</i>	1.111 µL / mL	---
<i>Footwear cleaning stations and hand disinfectants</i>	7 µL / mL	Disinfectant dispensers will be fulfilled with a solution of 0.7% v/v of Sanibacter® (7 mL for each L)
<i>Tyre baths</i>	2.5 µL / mL	For a footbath containing 1000 L of water, add 2.5 L of Sanibacter®. Repeat the operation every after heavy rain episodes

A further option, disinfectant mats (Fosse Ltd) (Figure 13 b,c) could be placed at the entrance and exit of roads crossing or bordering infected areas, to contrast the passive movement of the inoculum. The mats are filled with disinfectant solution (Blue, Arco® chemical group) with no runoff except when vehicles pass over. They will be accompanied by specifically designed, eye-catching signposts (Figure 13 d). These signposts: 1) detail the necessary steps to successfully clean vehicles and especially vehicle tires from inoculum whenever utilizing one of the roads crossing or bordering infected areas; 2) underline the severity of Phytophthora-infections and the damage caused by them in generally understandable terms, packaged in a meaningful call-for-action.

IV) Mobile cleaning kits: Easy-to-use cleaning kits including a disinfectant spray and brush to sanitize boots and other tools for those people (trekkers, workers, growers, forest technicians) frequently or necessarily interacting with infected areas (Figure 13a).

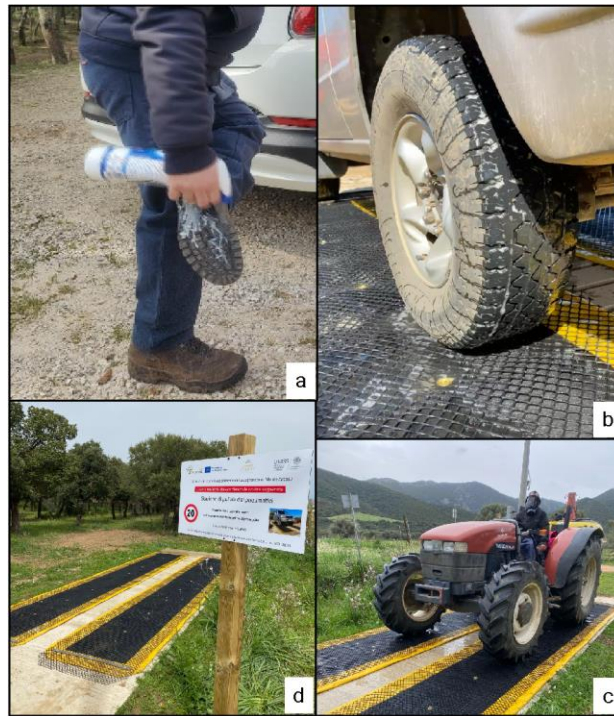


Figure 13. Mobile cleaning kits (a), Vehicles cleaning stations (b and c), signposting (d).

The entire implementation of the IMP can be seen in figure 14, which represents the activities carried out at Monte Arcosu farm (Sardinia, Italy)

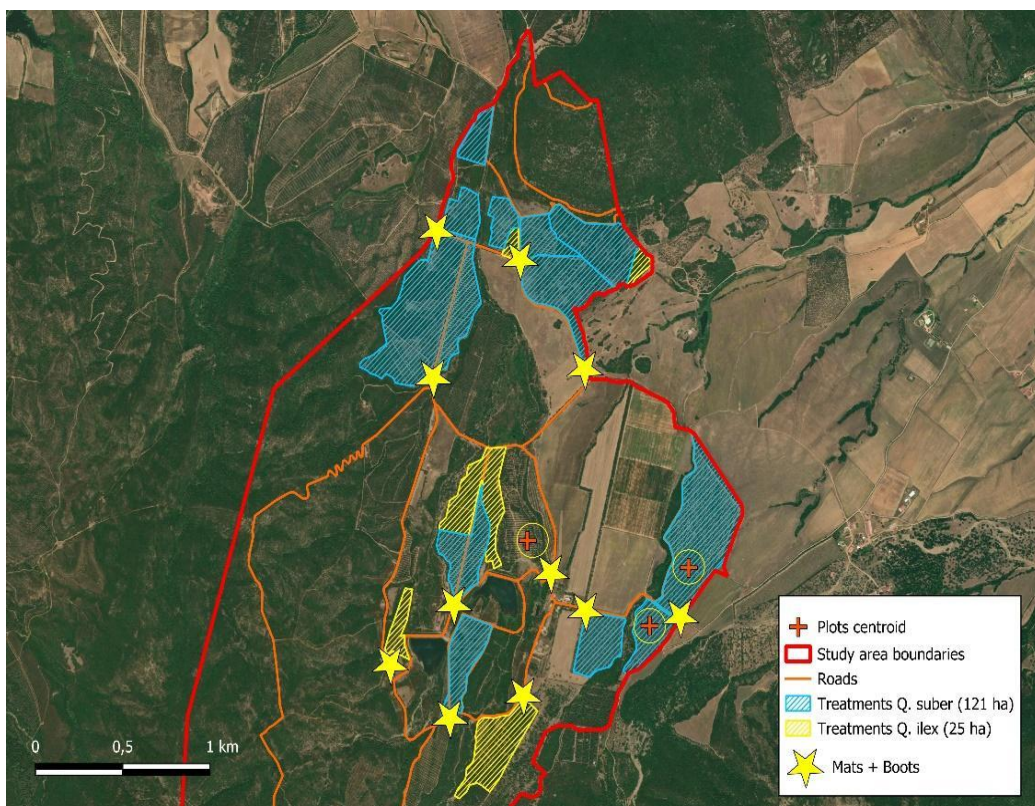


Figure 14. Maps of cork and holm oak plantation including the location of the vehicle cleaning station in Monte Arcosu farm.

5.1 Signposting

Hygiene measures will be accompanied by specifically designed, eye-catching signposts (Fig. 15).

These signposts have mainly the following objectives:

- Detail the necessary steps to successfully use the footwear cleaning stations
- Underline the severity of *Phytophthora infections* and the damage caused by this pathogen in generally understandable terms, packaged in a meaningful call for action.
- Warning about the presence of tire baths and the recommended speed at which they should be used.
- Indicating the area in which treatments will be carried out.



Figure 15. Signposting for hygiene measures and citizens awareness installed at the Demonstrative Area of La Tejera (Villaviciosa de Córdoba, Spain).

6. Best practices in evergreen oak “dehesas”

Finally, the management is considered a highly the relevant part of IPM protocols. Incorrect cultural practices are included among the inciting factors of decline. A series of measures listed below can be implemented for the management of holm oak “dehesas” regarding their relationship with oak decline and pathogen spread.

1. Keep managing the “dehesas”

The abandonment of “dehesas” leads to brush invasion and tree decay. The economic sustainability of “dehesas” should be at the center of the IPM protocols, as economically profitable “dehesas” are more likely well managed than poor ones. Extensive livestock and farming practices offering high-quality products are the best options.

2. Regularly monitor the health conditions of the holm oaks

Assess regularly tree defoliation and abundance of pests (insects and cankers). If defoliation starts to rise in a limited area, affecting several trees, or generalized decline and spread of pathogens come evident, promptly contact with a specialized forest or agronomist technicians for diagnosis and appropriate therapy. Keep in mind that early detection largely increases the efficacy of control measures and sensibly reduces the costs.

3. Carry out regular pruning

Pruning is functional to i) remove dead parts of the plant that are a source of pest and pathogen proliferation, and ii) stimulate vigorous vegetative and reproductive activity. Thus, it is recommended to carry out maintenance pruning every 3.5 years. The pruning must be carried out by expert arborists, in late winter or early spring, depending on the local conditions, but before the end of the winter rest period. The pruning must be carried out only in branches with a diameter above 15 cm and be careful to not cause damage to the tree due to wrong cut inclination or unintentional damage caused by incorrect tool use. Incorrect pruning or crafting practices would weaken trees inciting the decline process.

4. Avoid tillage with mouldboard plowing or deep plowing implements

These implements have negative effects on the decline process, both due to the damage of the oak root system, mainly its secondary roots, which are very shallow, and spreading infected soil. Scrub removal should be done by means that remove as little soil as possible, including chain brush cutters or hammers where possible. Planting should be done with shallow implements, if possible, avoiding tillage. These measures are very relevant when a disease focus is identified and *P. cinnamomi* diagnosis is positive.

5. Heavy machinery disinfection

In holm oak declining areas, the control of soil and vegetal material movement is critical. The inoculum of *P. cinnamomi* is present in the topsoil layers and most likely in the debris and the organic material of the soil. Thus, it can be easily transported with heavy machinery and implements. Therefore, disinfection of heavy machinery after cultural practices is mandatory. This disinfection can be easily carried out under normal conditions, using a 5% sodium hypochlorite solution (common bleach at 10%). A fumigation knapsack can be prepared with 1 litre of bleach and 9 litres of water, and applied to the running gear, dirty parts and implemented after the work is finished.

Disinfection can also be carried out with quaternary ammonium salts, adding an approximate quantity of 250 mL in 10 L to the fumigation knapsack.

When the soil is very wet if the accumulation of mud is relevant to the machinery, it is recommended to remove as much mud as possible before disinfection. This is also applicable to any vehicle entering the affected area. When the vehicles go off roads, the probability of accumulating soil and debris is higher, and, therefore, their disinfection must include the dirtiest parts as well as the wheels, so the tyre bath may not be sufficient.

6. Soil quality

Soil is pivotal in the health status of trees. Heavy soils, with low cation-exchange capacity, and very deficient nutrient soils are considered as predisposing factors of holm oak decline. Therefore, a soil analysis would be recommended in declining areas.

Limestone soil amendment can be positive when soil fertility is very poor. Limestone-rich soils are less prone to root rot, mainly due to their effect on the health status of the tree. When soil fertility is too rich or soils are deficient, a limestone amendment would be recommendable.

If the “dehesa” is regularly cultivated, it is also recommended to implement crop rotation with legume crops. In this case, avoid the use of lupin (*Lupinus luteus* L). This crop has been traditionally used in “dehesas” because of their nutritive value. However, it is not recommended in declining “dehesas”, as it can act as a reservoir of *P. cinnamomi* inoculum (is a non-symptomatic host).

Organic matter amendment should be carefully considered and used in several, very limited, occasions. *Phytophthora cinnamomi* can survive as saprophyte feeding on debris and rests of vegetal and organic matter in the soil. However, this pathogen is considered a bad competitor in saprophytic conditions. Therefore, high OM content in the soil can have a positive effect on the inoculum through the increase of microbial diversity and abundance, but it is not a mean to control the inoculum. Moreover, under low-organic matter concentrations, an increase in its content could even improve the conditions for pathogen survival.

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