



Integrated Pest Management protocol against chestnut ink disease

Part of deliverable D4.1 of workpackage 4

Large-scale application of new customized IPM protocols

Authors:	
Carmen Morales Rodriguez	Università degli Studi della Tuscia
Andrea Vannini	Università degli Studi della Tuscia
Romina Caccia	Canepina and Vallerano municipalities

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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 first phase of the project, different products were tested to mitigate the impact caused by *Phytophthora cinnamomi* on chestnut plants. This document provides the first release of a detailed IPM protocol for chestnut ecosystems 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, on 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, modification of cultural practices, and the use of resistant germplasm. Treatments, preferably based on environmentally safe molecules, are used only after the monitoring indicates they are needed according to established guidelines. Thus, pest control molecules are 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 that are unfavorable to the pest, also 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 reduce the dispersal of the pathogen; or soil fertilization treatment to increase the vitality of the plant.

III) Mechanical and physical controls

Mechanical and physical controls kill pests directly, block pests out, or make the environment unsuitable for them. 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, and the environment. In IPM strategies, the choice of selective molecules is encouraged to minimize the impact on non-target organisms and to preserve air, soil, and water quality.

3. Chestnut ink disease

Ink disease is a lethal disease of *Castanea* in Europe and the Southern USA. It causes root and collar rot in adult trees and seedlings in nurseries, plantations, and forests. Symptoms of the disease on adult trees include chlorotic leaves reduced in size, thinning of the crown, and immature husks remaining on the tree after leaf fall (Vannini and Morales-Rodriguez 2022).

The appearance of these symptoms corresponds to an advanced phase of the disease when most of the root system is already compromised (Vannini and Morales-Rodriguez, 2019) (Figure 1).



Figure 1. Suddenly dead plant with brown leaves still on the tree (A). Crown decline (B) Ink disease of chestnut: tree mortality and ecosystem degradation of a sweet chestnut (*Castanea sativa*) forest in Italy (C).

Together with the less aggressive *P. xambivora*, *P. cinnamomi* is the cause of ink disease of chestnut in Europe on *Castanea sativa*, and the main driver of the disease in Southern USA on *C. dentata* and *C. pumila* (American chinquapin). As for other soil-borne Phytophthora species, fine roots are firstly infected from where the pathogen starts the colonization of coarse and large roots. Flame-shaped dark necrosis is evident on the collar of the tree after debarking. On young trees with smooth bark, the necrosis is visible without debarking as depressed, slightly cracked areas at the base of the stem. The necrosis of the large roots induces the production of black exudates that stain the surrounding soil, especially during spring and fall, which is the name of the disease. Mortality occurs in

1–5 years depending on the age and size of the trees, and the *Phytophthora* species involved. Indeed, *P. cinnamomi* is more aggressive to chestnut than *P. ×cambivora* (Akilli Şimşek et al., 2019). Infected seedlings in nurseries or plantations undergo a rapid or gradual wilting of the leaves. In the root system, there is extensive necrosis of the taproot that extends to the lateral roots and up the stem for some centimeters. *P. cinnamomi* is the main cause of the disease in areas with mild winter while *P. ×cambivora* dominates where the average minimum temperature in winter goes below 5°C. However, the effects of climatic changes are dramatically modifying the distribution of the two species, and *P. cinnamomi* is gradually invading chestnut areas previously dominated by the less aggressive *P. ×cambivora*.

Epidemiology of the disease is strictly associated with climatic and soil conditions, landscape heterogeneity, and human activities. Chestnut areas with average annual precipitation above 1000 mm and limited summer droughts are particularly vulnerable to ink disease. Poorly aerated soils and intense human activities are also predisposing factors (Martins et al., 1998, 2007). The association of ink disease with natural drainage lanes and the network of forest and local roads was demonstrated.

Integrated management of ink disease 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 spread and dispersal of *Phytophthora*. Other variables include climatic factors that influence the prevalence of one of the two *Phytophthora* species over the other, 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-Rodriguez, 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 that 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 chestnut 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 X 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 2).

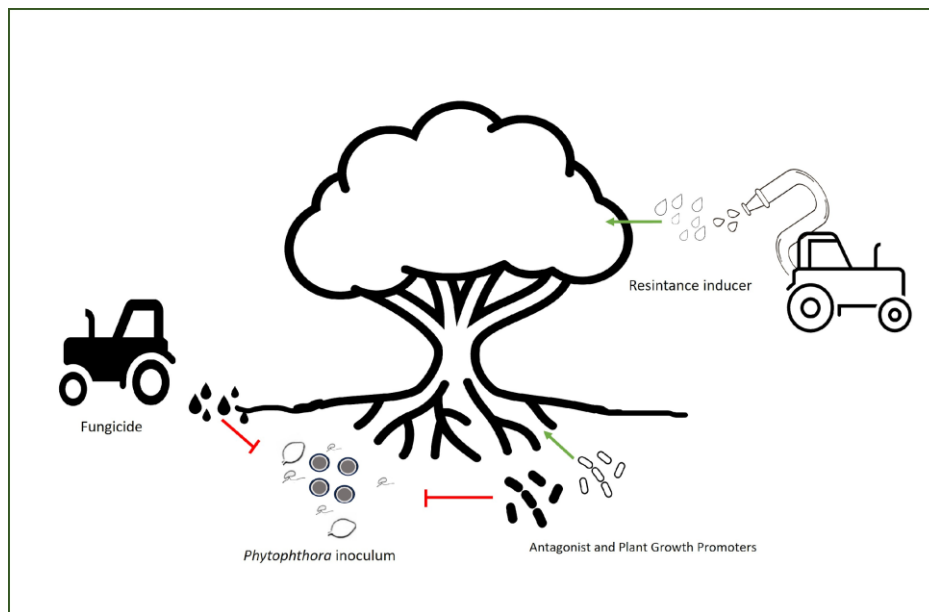


Figure 2. Holistic approach of treatment to mitigate the impact of *P. cinnamomi* in chestnut orchards.

4.1 Product formulation and dose

4.1.1 Reduction and mitigation 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, GLSs come into contact with myrosinases and are hydrolyzed 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).

Biofence FL (Nutrien Italia)

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 favours 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, consisting of a liquid formulary and brassica flour, to be combined and applied as a soil drench. Both formulations have been compared for efficacy on chestnut plants in mesocosm trials, resulting in the liquid formulation being the one with the best performance mitigation of the disease.

Composition:

Biofence FL is a two-component product, including liquid formulation and a flour derived from pellet of selected *Brassica* 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 3. Biofence FL. 10 liters of formulation + 5 KG of flour.

Preparation: (for 5 quintals of the product) (Figure 4)

- 1) shake the liquid formula to mix the components well and dilute it in a few Liters of water using a large container (2L of liquid formula + 8L water).
- 2) use the powder necessary for the treatment pour it into the special filter and then immerse it in container of previously prepared solution (1.8 kg flour).
- 3) leave the compound to infuse for at least one hour and not more than 4/5 hours. Shake the filter repeatedly 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 rinse it and therefore allow the isothiocyanate to come out and integrate into the solution.
- 5) the solution obtained must be brought to the volume necessary (5 quintals) to be able to distribute it with the watering can, making sure to spray especially the base of the plants and under the foliage area.



ATTENTION!!! USE WITHIN 4 HOURS OF PREPARATION



Figure 4. Preparation of Biofence FL. **A)** Prepare and measure the ingredients. **B)** Immerse the filter bag in the mixture of water and liquid solution. Leave the flour to infuse for at least one hour. During this time, shake the filter regularly so that all the flour is in contact with the liquid solution. **C)** After 1 h, rinse the filter bag and add the solution to a fumigation or fertilizer tank. **D)** To apply by treating the surface below the crown of the tree.

Mode of application and dosage:

- The product should be applied only in 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)
< 6	20
10 to 20	35
> 20	45

Only trees affected by illness should be treated by treating the surface below the top of the tree after the harvest or in wintertime 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.

4.1.2 Improving tree health through soil microbiome

This treatment is aimed to improve the plant physiology and to limit the habitat suitability for the pathogen through amendment with bioproducts based on plant growth-promoting microorganisms and antagonist. The inoculation of 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* (resistance inducer, competitors, parasitism) 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 (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).

Bactrium (Atens)

Bactrium® 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* species contained in Bactrium have been fully sequenced on our state-of-the-art platform 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 5. Bactrium, Atens®

Tricoten® (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 demonstrated a significant reduction of disease symptoms in holm oak 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 oak against *P. cinnamomi* because they were not included in the mesocosm trials. However, if their formulation is based in *T. atroviride*, it would be assumed that the effects will be similar.

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 6. Tricoten, Atens®

Mode of application and dosage:

- Dosage of Bactrium: 2L / ha
- Dosage of Tricoten: 1Kg / 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 can 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.

Century® Pro (BASF)

Century® Pro is a multipurpose fungicide based on potassium phosphonate, regularly authorized as an agrochemical. It is a systemic fungicide that rapidly penetrates the treated vegetation and is translocated in both an ascending and descending manner, resisting washout; therefore, it acts even in difficult conditions and represents a further guarantee of effectiveness for the farmer.

It is a real resistance inducer: the treated plants are protected from the inside thanks to the ability of the active substance to stimulate the plant's natural self-defense mechanisms. Furthermore, Century® Pro also has a direct action against pathogens. This "multisite" mode of action makes the product suitable for the construction of defence strategies aimed at preventing fungicide resistance phenomena.

The authorization of Century® Pro as an agrochemical, in addition to making it compliant with current regulations, guarantees a known and constant content of the active substance and co-formulants. This implies the absence of impurities and better residue management. The high quality of the formulation makes it simple to use and mix.

Composition:

100 g of product contains: Pure potassium phosphate 51.7 g (= 755 g/L) corresponding to 504 g/L of equivalent phosphonic/phosphorous acid. Co-formulants q. b. at 100 g.

DANGER STATEMENTS

EUH401 To avoid risks to human health and the environment, follow the instructions for use



Figure 7. Century Pro®, BASF

Mode of application and dosage:

To control chestnut ink disease, treat at a dose of 4.0 l/ha through foliar treatment before flowering.

Kalex Evo (Alba Milagro)

As an alternative to the Century® Pro in the case of organic farming. KALEX EVO is a fertilizer capable of nourishing and fortifying crops, making up for and preventing micro-deficiencies, and nourishing them in the most balanced way, it can keep them healthy and resistant. 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 its 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®, Milagro International S.p.A.

Mode of application and dosage:

To control chestnut ink disease, treat with a dose of 2 l/ha through foliar treatment before flowering. Do a second treatment after 20 days, while always avoiding the flowering period.

4.2 Period of application and chronogram

I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII

Biofence FL
Bactrium+Tricosan
Centrury Pro/Kalex evo

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.

Thus, hygiene measures must be considered 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 groves and along roads and trails crossing or bordering the groves, at least during flooding periods; (2) the disinfection of tires and shoes when at the exit from infected areas; (3) the management of superficial water flows by collecting and channeling infected water out of the chestnut area. 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.



Figure 9. Footwear cleaning stations (A). Mobile cleaning kits (B). Disinfectant liquid (Blu, Arco chemical group)(C). Vehicles cleaning stations (D)

In the case of hygiene measures, everyone's support is needed; in 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 chestnut areas of the municipalities of Canepina and Vallerano:

I) 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) who may be frequently or necessarily interacting with infected areas. (Figure 9. B)

II) Footwear cleaning stations: Commonly these stations include a brushing module to remove soil. In addition, they are either equipped with a spraying module to disinfect shoes or with a recipient for water and disinfectant integrated with the brushing module. (Figure 9. A)

III) Vehicle cleaning stations are an important preventive hygiene measure. Disinfectant mats (Fosse Ltd) will 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. 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. (Figure 9. D)

Figure 10 shows the location of the disinfecting mats within the chestnut orchards in the municipality of Canepina and Vallerano.

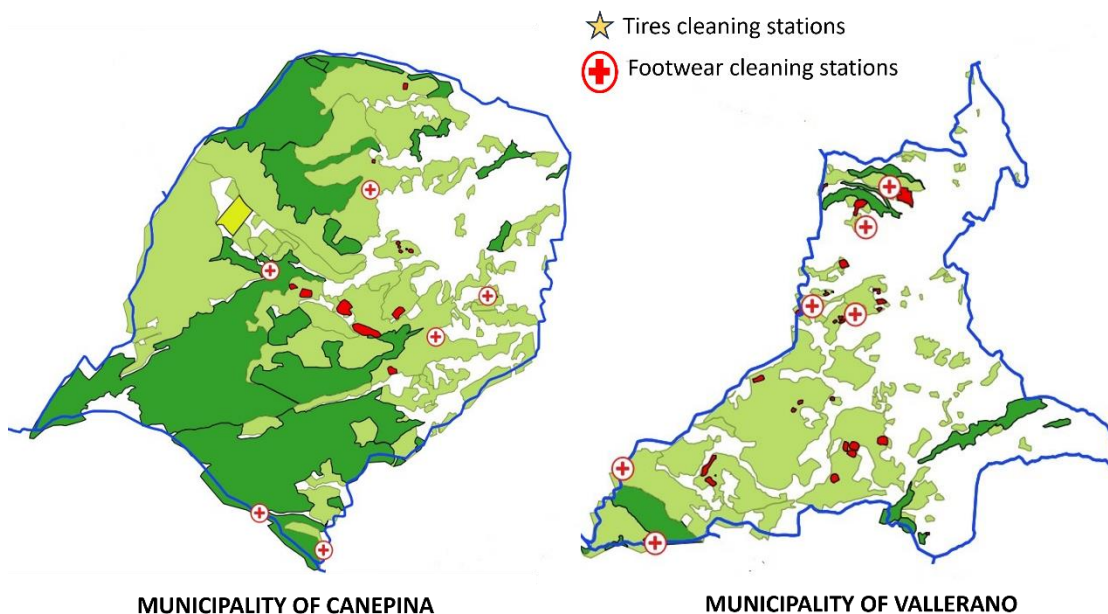


Figure 10. Maps of chestnuts areas including the location of the vehicle cleaning station in Canepina and Vallerano municipalities

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 and 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 considering also their composition, price, and availability in Italy, the product chosen to include in the demonstrative actions of FAGESOS is Blu (Arco chemical group) (Fig. 9.C).

Composition:

Quaternary ammonium salts

PH: 9,50+/-0,50 Density: 1,000+/-0,025 Kg/L

Mode of application and dosage:

The product is used by diluting it in water in a proportion of 1/200. In this way, a 1l package is enough for a total of 200 liters of disinfectant liquid. This can be used to fill mats from cleaning stations or to refill sprays for boot cleaning stations.

5.1 Signposting

Hygiene measures will be accompanied by specifically designed, eye-catching signposts (Fig. 11). 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.
- Warn about the presence of tire baths and the recommended speed at which they should be used.
- Indicate the area in which treatments will be carried out.



Figure 11. signposting for hygiene measures and citizens awareness installed in chestnut areas from Canepina and Vallerano municipalities.

6. Best practices in traditional chestnut orchards

A series of measures are listed that can be implemented for the management of the traditional chestnut orchards, with regard to the different aspects of chestnut cultivation and maintenance.

1. **Keep managing the chestnut groves** Traditional, centenary chestnut orchards must be considered, in a way, like any other fruit plantation. This is especially true in a scenario of global changes, where the lack of appropriate management can lead to irreversible decline and death. Furthermore, abandoned, or neglected orchards represent a potential host for pests and diseases that can pose a significant concern for neighbouring properties. Furthermore, traditional chestnut orchards provide a range of different ecosystem services extremely relevant for the preservation of soil, water, air quality, biodiversity, and primary and secondary products.
2. **Regularly monitor the health conditions of the chestnut tree** and promptly contact, if necessary, specialized agronomists for diagnosis and appropriate therapy. Keep in mind that early detection largely increases the efficacy of control measures and sensibly reduces the costs.
3. When strictly needed, traditional chestnut orchards must receive **phytosanitary treatments** to efficiently prevent and mitigate the impact of pests and diseases. **Always select eco-friendly molecules efficient against the target pest or disease but with no impact on the not-target biome.** Zero-impact protocols of treatment are now available to counteract the main phytosanitary problems of the chestnut trees (see above). The application of these protocols requires specific knowledge and the support of expert agronomists in the sector.

4. **Carry out regular pruning (every 2-3 years).** Pruning in traditional chestnut groves is functional to i) to remove dead parts of the plant that are a source of pest and pathogen proliferation; and ii) to stimulate a vigorous vegetative and reproductive activity. Thus, it must be carried out by expert arborists.
5. **Do not remove the pruning residues until June, setting them aside in the chestnut grove.** European chestnut areas since 2000 underwent a dramatic invasion by the Chinese Gall Wasp *Dryocosmus kuriphilus*. The invasion and impact were stopped following the release of the specific antagonist *Torymus sinensis*. However, the Chinese Gall Wasp cannot be eradicated and must be kept under control by favouring the activity of the antagonist. To do this, it is necessary to leave in the orchard on the ground the residual of pruning activities with antagonist colonized galls until June, to allow the adults *T. sinensis* to fly out from the previous year's galls, start its cycle on the new galls and counteract the infestation of the gall wasp.
6. **Organic fertilizers:** adequate nutrition plays a fundamental role, ensuring first and foremost both the quality and quantity of production. Moreover, there is no doubt that a healthy, well-nourished plant can more efficiently counteract the impact of biotic and abiotic stress factors, acquiring resilience. It is therefore important to fertilize the chestnut grove annually, preferring manure, manure, green compost, or organic mix, with a mineral fraction unbalanced towards nitrogen in the first 2-3 years in abandoned chestnut groves under-recovery, and, followed by a standard fertilization rich in phosphorus (and potassium, depending on the type of soil).
7. **Grafting** with productive varieties represents an important practice in traditional chestnut orchards carried out on old stumps or in the replacing of dead trees. The grafts are highly susceptible to chestnut blight fungus that represents the main cause of failure of the graft itself. The recommendation is to shift from graft types carried out with dormant scions such as whip and tongue grafts, or cleft one. These grafts are carried out in February when the inoculum of the chestnut blight fungus is still dormant. Bark graft types are carried out during late spring when temperatures are higher and the inoculum of the chestnut blight fungus is already dispersed and can easily infect the wounded tissues.
8. **Harvest the product promptly, daily.** The permanence of the fruits on the soil exponentially increases the risk of rotting and facilitates the pupal stage of harmful insects such as the European chestnut weevil.
9. **In areas with a high incidence of fruit rot, it is important to collect and burn the residues after harvesting,** because these pathogens overwinter in these materials.
10. **Carry out a correct post-harvest treatment of the fruit.** The most effective post-harvest treatments are flotation tanks to eliminate fruit affected by insects, and heat treatment in water (sterilization) at 50°C for 45'.

11. **Encourage the presence of oak species in the chestnut grove and in chestnut coppices**, because of the presence of native parasitoids the Chinese gall wasp. Therefore the presence of these species in the chestnut orchard must also be encouraged.

12. **Consider the chestnut grove not only from the point of view of fruit production, but also of multifunctionality**: the chestnut-growing territories represent an unmistakable landscape signature, they are rich in history, tradition, culture, and great beauty. Initiatives to valorise and typify the fruit and integrate it with the tourism, gastronomic, and cultural development policies of the area must therefore be encouraged.

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