

Innovation and Technology Transfer in the Treatment of Chestnut Blight in Portugal

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Forest4Eu [1], a European project funded by the Horizon Europe program, is a vital initiative. It is dedicated to uncovering and selecting pertinent information that showcases the wealth of knowledge and innovation emerging from the work of Operational Groups in Europe. These groups, particularly those focusing on forestry and agroforestry, are at the heart of the project. The information they provide will be disseminated in written or audiovisual formats, fostering a sense of connection and engagement across the participating countries. This work underscores the invaluable contributions of many Operational Groups to solving problems in the sectors of forestry and agroforestry, which often remain confined regionally or to their country of origin. It's a collaborative effort that you, as a member of the forestry and agroforestry community, are a part of. This article addresses the biological treatment of chestnut blight (*Cryphonectria parasitica*) in Portugal, based on a report from the Operational Group BioChestnut-IPM. The main geographical focus is the Trás-os-Montes region, where over 85% of Portugal's chestnut area is located. Within the scope of Forest4Eu, this work has been deemed highly significant for other producers and consultants in chestnut-producing countries, especially in the Mediterranean basin, providing practical solutions and insights for their work.

The relevance of this work on chestnut blight is underscored by the effective link it demonstrates between science and practice, providing important results for those working in this field. Such connections are common and important contributions of Operational Groups in various European countries, and they happen often. This leads to the hopeful prospect that these groups could receive robust support within the Forest4Eu framework, potentially even on a multinational scale.

The chestnut, in all its variants, is a cornerstone of the traditional forest species. It plays a crucial role in the productive, economic, social, and environmental aspects of the regions it inhabits. Therefore, it is imperative that we focus on improving its health status and attractiveness as the foundation of forestry or agroforestry operations.

Materials and methods

This article is based on the report "Manual of Good Practices for the Biological Treatment of Chestnut Blight (*Cryphonectria parasitica*) in Portugal," published by the National Center for Dry Fruit Competencies. We aim to highlight this chestnut disease's critical aspects and suggest future work directions.

Chestnut Blight: An Overview

Chestnut blight (Figure 1) is a well-known disease in phytopathology and a classic example of an introduced and exotic phytopathogenic fungus that nearly eliminated the American chestnut (*Castanea dentata* Marshall - Borkh) throughout its natural range. Introduced to Europe in 1938, it has shown high virulence in the European chestnut (*Castanea sativa* - Mill.).

The disease spread rapidly wherever it appeared, causing the death of many thousands of chestnut trees. In Portugal, the disease has reached epidemic levels since its introduction in 1989 and is present in all producing regions, causing high mortality rates in chestnut trees.

Asexual spores (conidia) form in pycnidia with a 100-300 µm diameter under high humidity conditions and are released outside, enclosed in a gelatinous substance called "cirro". Conidia are the vegetative propagules of new infections or can function as male gametes in sexual reproduction. They are dispersed by rain and carried along stems and branches, where they cause new infections. If they reach the soil, they can remain viable for a long time and cause infections at the base of trees. Birds, insects, mites, and strong winds can transport conidia, spreading the disease far from the initial focus.

Pruning and grafting of trees can also contribute to spreading the disease through the fungus mycelium, which is directly transferred via cutting tools. In the case of cuts, disinfecting the tools can enhance disease containment; in the case of grafting, these may become unviable due to infection with *C. parasitica*, as there is no vegetative material with sanitary guarantees for grafting. The effect of CHV1 strains on the grafting process is unknown (Gouveia et al., 2022, p. 6).

Common disease symptoms include reddish branches, yellow-orange necrosis on the bark, and variable-sized cracks. Due to the rapid disease progression, branch death occurs shortly after, with necrotic and dry leaves adhering to the branches for some time. Note that some symptoms may (apparently) be similar to other cortical tissue diseases with less severe impacts - hence the importance of diagnosing the disease before starting treatment.

Biological Treatment through Hypovirulence

After several years of implementing legislative measures to mitigate the disease's devastating effects and control it, these measures proved ineffective. There was an urgent need for alternative, more efficient control measures with significant adherence from producers. This led to the adoption of biological treatment strategies, such as hypovirulence, to effectively manage chestnut blight.

In Europe, where chestnut blight has existed since 1938, biological control based on applying hypovirulent strains of *C. parasitica* (hypovirulence) is considered the most effective means of control. The European Food Safety Authority (EFSA) also recommends this method when the disease is present and naturally occurring hypovirulent strains are absent (EFSA, 2016). Hypovirulence is a biological mechanism that heals cankers, leading to the complete recovery of diseased trees, occurring naturally in chestnuts.

Hypovirulence (reduction of virulence) is mediated by complex biological and molecular processes involving the parasitic fungus (*C. parasitica*), the biological control agent (the virus *Cryphonectria hypovirus 1* - CHV1), and the chestnut tree (*C. sativa*). The process is also dependent on and influenced by environmental conditions, the introduced population of the parasitic fungus, and the characteristics of the hypovirus introduced (or naturally present) as the biological control agent.

The biological control method using hypovirulence, with the application of hypovirulent strains of *C. parasitica* (CHV1 strains), has demonstrated high efficacy in treating chestnut blight in Portugal (Figure 2), leading to the healing and complete recovery of treated chestnut trees.

The fundamental steps for the application of the biological treatment for chestnut blight based on hypovirulent strains of *C. parasitica* (CHV1 strains) include:

- a)** Understanding the genetic structure (vc type) of the virulent *C. parasitica* population present at each location.
- b)** Obtaining, identifying, and characterizing hypovirulent strains of *C. parasitica* compatible with the virulent strains of the fungus at the different locations to be treated.
- c)** Producing the biological agent in the laboratory and ensuring the reproducibility of characteristics during the "scale-up" of the production process.
- d)** Test and validate the application methods and timings for therapeutic treatment of the blights.
- e)** Obtain official application authorizations (in Portugal, DGAV is the competent authority) and comply with legal requirements and procedures resulting from the official authorization for use.
- f)** Train applicators and disseminate the new method and product.

The actual treatment is carried out by applying the bioproduct DICTIS, which was developed explicitly for chestnut blight in Portugal. The bioproduct can be used throughout the chestnut's vegetative activity period (March to November) to treat active blights. It is crucial that the bioproduct formulation is adapted to the genetic structure of the fungal population present at each location.

The DICTIS bio-product can be applied via drilling or brushing. It should be applied to the edge of the blights, in the healthy area, but as close as possible to the diseased area. Brushing is applied over the entire affected area after scarifying the necrotic tissues. The necrotic tissues of the blights should not be removed from the chestnuts and should remain on the tree throughout the healing process (Gouveia et al., 2022, p.14).

During the application of this biological treatment, two essential aspects must be considered: i) recognizing that the tree's response time may take several months and ii) monitoring the treatments and making necessary adjustments.

Blight healing and tree recovery, including its productive potential, were observed in most treated chestnuts.

Key Aspects of the BioChestnut-IPM Operational Group's Work

To carry out biological treatment using hypovirulence, it is essential to understand the legal regulations guiding such practices and their application in the field. The formulation of the biological control agent adapted to each location, and the ability to analyze the genetic structure of the parasitic fungal population and monitor effects must be considered to optimize the method and ensure its efficacy, avoiding resource wastage.

Moreover, the collaboration of various efforts and entities is crucial. In this case, there was cooperation among educational and research institutions (coordinated by the Polytechnic Institute of Bragança, and including the Polytechnic Institute of Viana do Castelo, UTAD, INIAV.IP), representatives of farmers, individual producers, public land management entities (municipalities), and a central public entity (Ministry of Agriculture - DGAV) responsible for regulation and supervision in the use of plant protection products.

The implementation of Operational Groups, as demonstrated here, shows their effectiveness but also highlights the need to promote the continuity of this type of work because: i) the production circumstances and population structure of the parasitic fungus and type of hypovirus may change, and ii) the introduction of plants with other vc types alters the parasitic fungal population structure and consequently reduces the method's efficacy. Therefore, there is a need to maintain ongoing care for the chestnut orchards.



Figure 1 - Trees affected with Chestnut Blight.



Figure 2 - Blight healing after treatment: (a, b) two years after treatment; (c) 3 years after treatment.



Figure 3 - Castanea sativa tree.

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<https://www.calameo.com/read/006243536f10c5b3dad80>

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Further information

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