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# Management of Chestnut Blight in Greece Using Hypovirulence and Silvicultural Interventions

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**Abstract:** Sweet chestnut (*Castanea sativa* Mill.) is an important tree for Greece. The invasive fungus *Cryphonectria parasitica*, which causes chestnut blight, was first found in Central Greece in 1963. It has since spread all over the country, significantly reducing the national annual nut production. The increasing decline of forests and orchards due to the disease led to a project in 1995, which aimed at studying the feasibility of applying biological control. A prerequisite study of the existing vegetative compatibility types of the pathogen showed only four, and their distribution was mapped. A pilot project (1998–2000) that consisted of clear cutting heavily infected coppice stands and introducing hypovirulence to the remainder was implemented on Mt. Athos on a 7000 ha sweet chestnut forest. Two evaluations (in 2003 and 2011) revealed that hypovirulence was established in the sweet chestnut forests and spread more or less homogeneously. A nationwide project introducing hypovirulence to 29 counties was implemented in two, 3-yr-periods 2007–2009 (17 counties) and 2014–2016 (12 counties). The new evaluations showed that hypovirulence spread profoundly and forests and orchards started recovering. The appearance of natural hypovirulence cannot be predicted. Introduced hypovirulence and silvicultural interventions can be used to manage the disease. It is the responsibility of the forest/orchard manager to decide whether to wait for appearance of natural hypovirulence, or to introduce it for a faster decline in disease.

**Keywords:** disease management; biological control; chestnut blight; *Cryphonectria parasitica*; hypovirulence; silvicultural interventions

## 1. Introduction

Sweet chestnut (*Castanea sativa* Mill.) is an important tree for the European countries in the Mediterranean region. The cultivation of sweet chestnut is particularly important for Greece, as it is a key crop for the economy of mountain areas. It is cultivated in orchards for its edible nuts and in coppice forests for its highly valued timber.

Pollen analysis has shown that Northern Greece had chestnut trees as early as the 10th century B.C. [1], while more recent evidence has shown that sweet chestnut was cultivated in North-Eastern Greece from 2100–2050 B.C. [2]. The migration of human populations, particularly the Greeks and Romans, appears to have played a fundamental role in the spread and cultivation of sweet chestnut, both for its fruit and wood. Sweet chestnut was carried west, initially to the Greek colonies in Italy [3] and later all over the Mediterranean coast.

In Greece, sweet chestnut is found all over the country; from the northernmost border down to the island of Crete. A decline in sweet chestnut cultivation was experienced in the second half of the 20th century in Greece, mainly because of:

- The migration of the mountain people to cities, and consequently, the abandonment of the orchards;

- The rapid spread of chestnut blight, which devastated orchards and coppice forests alike;
- The lack of policies that would encourage and support chestnut growers.

Chestnut blight caused by *Cryphonectria parasitica* (Murr.) Barr has spread all over Greece since 1963, when it was first recorded on Mt. Pelion [4,5]. The disease was quite destructive in many areas, hastening the migration of people who could no longer make their living by chestnut cultivation [6]. As a result, the national, annual chestnut production was reduced from 18,000 tons in the 1960s to 11,000 tons in 2005.

Analyzing the American and European experiences, biological control of *C. parasitica* using its mycoviruses was considered the most promising method to remedy the problem and restore the chestnut orchards and forests [7–11]. Biological control projects have been previously implemented in France and Italy [12,13] with encouraging results.

Knowledge of the vegetative compatibility types (vc types) of the pathogen and their geographic distribution in a country are crucial for the successful application of biological control [14,15]. Thus, before any planning of the biological control of chestnut blight, the identification of the existing vc types, their distribution and mapping is absolutely essential [16,17].

Therefore, the first goal of an attempt to save the sweet chestnut in Greece was to examine the feasibility of applying biological control by using hypovirulence on a national scale. A second goal would be to organize the actual application of biological control, in case it was shown that such a project was feasible and effective.

## 2. Preparatory Tasks before the Application of Biological Control

Under the pressure of an increasing decline in chestnut production due to chestnut blight, a project aiming to study the vc types of *C. parasitica* and their distribution in the country was set out in 1995. Sampling was carried out in all 29 counties where chestnut grows, either naturally as old growth and coppice forests, or in orchards. In areas where both natural coppice forests and orchards were present, samples were taken from each. Cankered trees were selected more or less evenly throughout the area of each population [6].

The isolation of the fungus from bark samples, identification of vc types and conversion to hypovirulence using the CHV-1 mycovirus (Italian subtype) were conducted following standard techniques [6,7,9]. CHV-1 had occurred spontaneously in isolated spots on Mt. Pelion in Central Greece, where the disease was first detected in 1963 [18].

In 1988, a major threat to the chestnut coppice forests of Mt. Athos, a UNESCO protected site, triggered the need for fast intervention. A study was conducted aiming to apply biological control against chestnut blight. Extensive sampling in the 7000 ha forest revealed that there was only one vc type of the fungus *C. parasitica*, that of EU-12. A work plan was elaborated, consisting of the clear cutting of severely infected compartments and field inoculations. All felling operations were performed in 1998 during the first year of the three-year project. A compatible hypovirulent inoculum was produced using the CHV-1 mycovirus isolated from Mt. Pelion. The field inoculations were conducted in 1998, 1999 and 2000 by trained personnel. Approximately 20,000 accessible cankers were treated, which were more or less evenly distributed throughout the chestnut forest. This project worked as a pilot trial for the next, nationwide assignment.

When chestnut blight has spread all over a country, circumstantial and locally applied measures are ineffective. Action should be taken simultaneously on a national scale if there is to be any hope for fast combat of the disease and the rehabilitation of the chestnut forests and orchards. After the collected information showed that hypovirulence could be used to manage the disease, the decision was made to introduce hypovirulence on the national scale through artificial inoculations in two, 3-yr-periods 2007–2009 (17 counties) and 2014–2016 (12 counties). The spontaneously expressed hypovirulent (hv) strain with the CHV-1 subtype I (Italian subtype) virus from Mt. Pelion was used to prepare the hv inoculum for the tree inoculations, and thus spread hypovirulence all over the chestnut growing areas.

The transmission of viruses by mycelial anastomosis from hypovirulent to virulent strains is feasible only if the two strains fall within the same type of vegetative compatibility (vc type) [12,15,19]. Therefore, the inoculum was specific for each county and compatible with the local vc types of the fungus *C. parasitica* according to a vc type distribution map that had been previously elaborated [6]. All the inoculum was prepared at the Forest Research Institute on a commercial level, but with laboratory care [20]. In counties where more than one vc type was recorded, the Institute supplied a blend of inoculum of the corresponding vc types at the proportion found.

Such a large-scale operation involving chestnut growers, foresters, laboratory technicians, research scientists, laboratory work and field application, and which depends on time restrictions, requires flawless organization and timing. Before the application of biological control in the field, certain prerequisites are fundamental; the most important being a national survey in order to identify all the existing vc types of the pathogen. After this first step, a good deal of other work has to be organized, making the preparation of an "Integrated Biological Control Plan" aimed towards the management of the disease essential [21].

*Integrated Biological Control Plan*

An Integrated Biological Control Plan aims to give the manager all the necessary information in order to successfully organize the introduction of hypovirulence in chestnut areas where it has not already appeared naturally. Furthermore, it aims to recommend sylvicultural/horticultural interventions in order to reduce the disease occurrence potential, slow down the disease spread and give time for the introduced hypovirulence to establish, spread and dominate the virulent strain/s in the field.

When the Integrated Biological Control Plan refers to a national scale project, then it should comprise a number of Biological Control Plans that refer to smaller areas such as regions, counties, municipalities or individual forests [21].

The objectives of an Integrated Biological Control Plan are:

- To assess the health condition of the forest stands or orchards and estimate the magnitude of the damage;
- To map the exact position of the disease centers for easy access and fast inoculation by the field teams;
- To estimate the approximate quantity of inoculum needed for each particular area;
- To estimate the cost;
- To deal with any other organizational details.

The health condition of the forest stands and orchards was calculated after a statistically adequate sample of trees along transects was determined. Three health categories were established:

- *Stable* when the disease severity was found to be 0%–10%;
- *Unstable* when the disease severity was found to be 11%–40%;
- *Critical* when the disease severity was found to be >50%.

A disease severity between 40% and 50% was handled by considering other criteria such as the age of the trees, location, effects of clear cutting (if any), etc. For example, infected stands on steep slopes that protected the soil from erosion could be assigned a 40% level instead of 50% so that they remain, instead of being clear cut. Similarly, stands near sylvicultural maturity could be kept instead of being clear cut.

## 3. Results

The field sampling and the bench work revealed that only four vc types existed in Greece: EU-1, EU-2, EU-10, and the dominant EU-12, which accounts for 88% [6]. The perfect (sexual) stage of the fungus was never found. As (a) it was previously established that the fewer vc types in a wide area, the more effective the control may be; (b) localized, natural hypovirulence had previously occurred in Greece; and (c) the pilot project in Mt. Athos produced positive results and valuable experience, the decision was taken to apply biological control on a nationwide scale using Greek mycoviruses. An Integrated Biological Control Plan was compiled.

Wherever possible, forest stands or orchards in *Critical* condition were clear cut. The timber produced was removed from the forest as soon as possible. As the rotation in sweet chestnut coppice forests in Greece is 20–25 years, most of the produced wood products were fence or vineyard poles, rather than construction timber. All timber was absorbed into local markets without any particular disturbance in prices. No intervention was suggested in *Stable* stands or orchards, but their health condition was closely observed. Tree canker inoculations were applied in *Unstable* stands and orchards to trees aged from 5 to 18 years. The logic behind selecting young trees was that chestnut bark becomes rough and cracks longitudinally after the age of about 20 years. During canker inoculations the holes are made in the periphery of the canker at a distance of 3–4 cm away from the canker edge. The hypovirulent strains can establish successfully only on healthy bark and the edge of the cankers can be seen only in young trees with smooth bark.

Approximately 170,000 accessible cankers were inoculated for three consecutive years, in each of the two periods dating from 2007–2009 and 2014–2016. All field inoculations were carried out by trained foresters and forest workers under the supervision of the Forest Research Institute and Forest Service regional foresters [6]. The entire project was financed by the Forest Service, which meant that the chestnut growers did not have to cover any of the cost.

Several evaluations after the end of the inoculations showed that the introduced hypovirulent strains had established on the inoculated trees, healing the particular cankers, and that they had also started to disseminate to and heal non-inoculated cankers as well [20].

The effect of the management type (orchard vs. coppice forest) was significant for the dissemination of hypovirulence. The spread of hypovirulence was faster in coppice forests than in orchards [20]. In clear cut stands, a rather heavy infection was observed in the first 2–3 years in injuries along the densely sprouting shoots because of crowding. The initial feeling of disappointment was followed by relief, however, when the fatal cankers gradually converted to healing cankers by the already-established hypovirulence.

Forest managers and individual chestnut growers, as well as the official government of Mt. Athos, happily state today that chestnut blight in Greece is now history, and that they have returned to cultivation as before the occurrence of the disease.

## 4. Discussion

Chestnut is a multipurpose tree cultivated in commercial plantations for nuts and in coppice forests for timber. It combines rural economic importance with important ecological benefits, such as protection against fire and erosion, an excellent habitat for biodiversity and positive effects on climate and recreation. When a disease like chestnut blight enters a country, it is certain that sooner or later it will spread, causing unbearable loss. In several European countries, spontaneous hypovirulence appeared approximately 30 years after the initial entrance of the disease. It is a hard decision for administrators whether to wait for natural hypovirulence to occur and naturally spread, or to implement a costly biological control project after specialists recommend its feasibility.

The project implemented in Greece showed that even though spontaneous (but localized) hypovirulence may start occurring, its spread may be slow and dramatically different from area to area. Impatient chestnut growers would use any means to seek a fast resolution.

Crucial reasons such as (a) the decline of the Greek national chestnut production due to the disease; (b) the socio-economic impact due to property loss and finally the immigration of chestnut growers from their mountain villages, resulting in the abandonment of their orchards; (c) the devastation of sweet chestnut orchards and coppice forests; and (d) the existence of only four vc types of the pathogen, contributed to the consideration of managing the disease by applying biological control along with silvicultural/horticultural interventions.

The design of a successful inoculation project on a nationwide scale and the preparation of the hypovirulent paste for diverse counties with different vc types of the pathogen is difficult, painstaking and requires a good deal of scientific experience and long preparatory work [6].

The Integrated Biological Control Plan proved highly useful. The financial support of the Forest Service encouraged chestnut forest and orchard owners to participate in the project. As a consequence, all interventions were planned and implemented at the same time and at the right time in all chestnut-producing counties. Cankers extend fast in spring and early summer. The inoculations started on 1 May and lasted until 15 July, so that the virulent strains of the extending cankers hit the hypovirulent strains that were inoculated around the cankers within the growing season. The conversion of the virulent strains to hypovirulent strains and the subsequent production of pycnidia and spores resulted in the dissemination of hypovirulence.

During the evaluation of the project, it was found that there was a remarkable difference in the speed of disease decline from area to area. The age of the trees and horticultural practices may significantly affect the establishment and dissemination of hypovirulence. The spread of hypovirulence was faster in coppice stands than in orchards. As observed in Italy [22], we found that in orchards the results were affected by the horticultural techniques applied by the farmers and the care involved. Interventions in orchards such as grafting, pruning and wounding the trees with tools and machinery hampered the control process. In Mt. Athos we saw cases where silvicultural salvage operations in coppice stands aimed at reducing the disease potential by removing infected trees where no sign of natural hypovirulence had been detected, actually resulted in a sharp increase in the disease and high tree mortality. This was mainly because of tree wounding during the felling and extraction. However, when there is natural or introduced hypovirulence, such wounded trees and their initially lethal cankers gradually convert to healing ones [20].

The dissemination of hypovirulence is a dynamic process that evolves over time. As hypovirulence gradually increases, virulence is expected to be reduced. Consequently, the results of the biological control of chestnut blight are not seen for the first few years after the inoculations. In fact, they cannot be seen by the chestnut growers even several years later, because the growers cannot easily distinguish lethal cankers from healing or even healed ones. After the inoculations and the establishment of hypovirulence, the cankers cannot be visually identified as lethal or healed. There are cankers in the process of conversion which cannot be distinguished, even by an experienced eye. The main criteria are that the branch above a large lethal canker will soon die, and there will be a few adventitious shoots growing on the lower side of the canker. In healing and healed cankers, the adventitious shoots will start dying and the branch will show signs of recovery. Sweet chestnut growers ignore the overall health status of their trees and tend to remove the limbs of the trees every time they observe cankers. As a result, they end up with poorly shaped trees. In addition, by removing the superficial cankers they actually reduce the production of pycnidiospores carrying the viruses, which would eventually remedy the disease. It is very important to educate chestnut growers in all the treated counties about what to expect, how to recognize the identity of the cankers and what to do. For example, significant arguments erupted in several counties between chestnut growers who owned orchards with old trees and the field crews. When the latter refused to inoculate their trees, the chestnut growers responded fiercely and considered the act as discriminatory against them. It is crucial therefore to teach growers well in advance how biological control works and encourage them to have confidence in the science, the scientists applying it, and the importance of patience.

## 5. Conclusions

We reached the conclusion that when biological control against chestnut blight is implemented in such a way that all involved parameters (such as the identification and mapping of vc types, the preparation of hv inoculum, using trained personnel for field work, and the continuous monitoring and education of chestnut growers) are thoroughly considered, then the results should be expected to be satisfactory.

Systematic inoculation in a dense network of cankered coppice sprouts or orchard trees can contribute to the establishment of hypovirulence and greatly enhance its spread in comparison with natural, locally-expressed hypovirulence. It is the responsibility of the forest manager/orchard owners to decide whether to wait for natural hypovirulence to occur and spread spontaneously or to introduce hypovirulence for faster results [20].

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