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# Hygrothermic treatment of chestnut logs infected with *Cryphonectria parasitica*

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**Summary.** Due to the reduced availability of large-sized chestnut logs in Europe, many European timber industries currently get their supplies from non-European countries, mainly from the Caucasian region, which are often not immune to chestnut blight. Given the high risk of introducing new virulent strains incompatible with local hypovirulent ones, the European Union regulation requires that chestnut logs, imported from so-called "third party" nations where *Cryphonectria parasitica* is present, reach the European boundaries bark free: this prevents the production of veneers, which are highly remunerative, but whose first workmanship phases require barked logs. Following a multilevel investigation, the authors propose a stem-flow protocol that can devitalise the parasite in barked logs while preserving the commodity characteristics of the wood, through a fast, simple and low-cost treatment, that can be performed at the European borders whenever *C. parasitica* is or might be present.

**Key words:** hygrothermic treatment, European chestnut, chestnut blight, *Castanea sativa, Cryphonectria parasitica*.

## Introduction

Chestnut blight is caused by the wound parasite *Cryphonectria parasitica* (Murrill) Barr (Barr, 1978). Due to its easy diffusion through conidia and spores transported by wind, rainstorms, insects and other animals (Sharf *et al.,* 1981; Russin *et al.,* 1984), the fungus can easily be transmitted to uninfected portions of the same tree and move to the neighbouring chestnuts, spreading the disease in the original host and in the stand. In mixed forests it also colonises other plants (i.e. *Quercus, Alnus, Carpinus, Ostrya*) where, although not causing severe damage, it increases its inoculum potential (Turchetti *et al.,* 1991). Once a natural or artificial wound has been reached and colonized, the fungus grows in and under the bark, killing the cortical and cambial tissue around twigs, branches or stems, and drying up the distal portions. The disease gradually moves in a basal direction also parching the developing shoots and consequently giving the tree a multiple-stemmed shrub shape (Anagnostakis, 1995; Milgroom, 1995). Fortunately, in Europe this typical disease development can be slowed and stopped by less dangerous, hypovirulent strains of the parasite, transfer this feature to vegetatively compatible typical strains through hyphal anastomoses (Grente, 1965). Due to both the natural and artificial spread of hypovirulent strains, chestnut blight in Europe is gradually becoming

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an endemic disease (Bissegger *et al.,* 1997; Robin and Heiniger, 2001).

Currently, the greatest risk of disease recrudescence is through the introduction of new virulent strains incompatible with the local hypovirulent ones. This is the main reason why European Union regulations list *C. parasitica* amongst the quarantine parasites and forbid the import of logs with rhytidome in European *protected zones* from non-European countries where the parasite is known to be present (European Council, 2000; 2004). This compels importers to replace the production of veneers, which is highly remunerative but requires barked logs, with other types of products, causing a loss of the raw material market value that may reach 70% in plant production.

Since chestnut blight often occurs in the countries from which the main imports of large logs currently arrive (especially the Caucasian and peri-Caucasian regions, Pridnya *et al*., 1996), this study evaluated the possibility of developing a stem-flow protocol that will devitalise the pathogen in logs with bark directly on lorries at European Union points of entry through a fast, simple and low-cost hygrothermic treatment that preserves the commodity characteristics of the wood.

## Materials and methods

To set treatment parameters, preliminary investigations examined both the temperature and the exposure time required to devitalise fungal propagula in a wet environment: for this the heating dynamics were studied on steamed chestnut logs and their moisture level (Hunt and Garrett, 1967) was measured after treatment.

#### Laboratory trials on *C. parasitica* cultures

Laboratory trials were performed using five *C. parasitica* from infected chestnut logs that had arrived from the Krasnodar region (Caucasus, RU), the high pathogenicity rate of which had previously been verified through artificial inoculations on 4-year-old chestnut trees (*Castanea sativa* Mill.). For each strain, 100 discs (10 mm diam.) of colonised substrate were taken from the perimeter of colonies grown on potato dextrose agar (PDA) at 22±2°C, with a 12 hour photoperiod for 30 days. The discs were placed on PDA in the centre of 100 Petri dishes (12 cm diam.) and incubated under the same conditions for 30 days. The open dishes were then placed vertically in a thermostatic steamer modified to maintain a constant air temperature, to allow the free flow of steam and avoid condensation pools. In this way 20 treatments were arranged from the following combinations of temperature and exposure times: 40, 50, 60 and 70°C; 0, 15, 30, 45 and 60 min. Each treatment had 5 replicates. At the end of the treatment period, transplants were made from all the material to PDA to re-isolate the fungus under the same incubation conditions as above. Results were statistically treated with the $\chi^2$ test ($P$=0.05).

#### Trials on wood logs stem-treated in the laboratory

Heating dynamics in the wood were studied on 9 healthy logs (length 200 cm, median diameter 30–32 cm) with comparable rhytidome types. They were laid out in parallel on a beaten earth yard 1 m apart, and raised from the ground with square 15 cm section wooden beams. In order to measure the longitudinal hygrothermal conduction on three logs, 5 holes (1.2 mm diam.) were bored at 0, 20, 40, 60, 80 and 100 cm from one end to a depth of 30 mm, which is the safe limit within which the parasite can be found according to the EU regulations. A copper and constantan probe (1 mm diam.) was placed in the holes, sealed with heat-resistant silicon and connected to a thermometer with a multi-channel microprocessor. Using the same methods, radial hygrothermal conduction was determined on 6 logs by connecting the probes at depths of 1, 3 and 4 cm along the median log circumference. A PVC flexible hydrant hose (6" diam., 6 bar), with the internal rubber sheath removed and connected to a boiler (630,000 kcal $h^{-1}$, 150°C at the outlet), was placed beneath the logs, which were then wrapped in heat-resistant PVC tarpaulin (Plyvil SL, thickness 0.25 mm) held to the ground by covering the edges with soil. The treatment, performed at 22±1°C air temperature, consisted of steaming at a constant 99.5°C for 180 min, during which the temperature reached by the different probes was registered every 10 min in order to identify the time necessary to reach the minimum temperature for mycelium devitalisation observed in the *in vitro* trial. Logarithmic

regressions were then performed on the collected data to obtain the temperature curves and relative confidence intervals ($P$=0.01).

The moisture level of the logs was determined by weighing 18 wood samples (2 per log, length 1 cm, 0.5 mm diam.) collected from the middle portion both before and after steaming, using a Pressler auger. The fresh weight of the cylinders was compared with the dry weight, obtained by drying at 160°C for 120 min.

**Trials on wood logs stem-treated on lorries**

According to the results obtained from these trials, stem-flow devitalisation was performed at 24±2°C air temperature on infected logs loaded on lorries in the port of Venice. Among the available barked logs with visible pycnidia that had just arrived from the same region and from which the five strains used in the *in vitro* trial had previously been isolated, the 15 most representative of a typical supply shipment were selected (length 350–450 cm, 60÷80 cm diam., cracked and partially lacking the rhytidome). From each of these logs, 5 samples (l×l×h, 10×10×5 mm) of sub-cortical tissue with typical *C. parasitica* mycelium were removed before and after the trial, placed on PDA and incubated at 22±2°C with a 12 hour photoperiod for 30 days.

The logs were loaded on 3 lorries (5 per lorry, including at least two with 80 cm diam.) in a pile with other logs to simulate a realistic situation. The flexible PVC hose connected to the boiler was placed around the pile and the whole was covered with a heat-resistant PVC tarpaulin as described above. The edges of the tarpaulin were fixed to the body with clamped wooden boards.

The treatment cycles were set through theoretical extensions of both the experimental conduction curves and previously obtained temperature gradients, and therefore performed on the 3 lorries for 120, 150 and 180 min respectively. To avoid commercial damage to the logs no thermal probes were used.

## Results and discussion

**Laboratory trials**

Laboratory trials performed on *C. parasitica* strains showed that steam treatment at 60°C for 15 min devitalised both mycelium and pycnidia.

Studies on heating dynamics in wood revealed, as expected (Giordano, 1983), that temperatures in a longitudinal direction decreased logarithmically depending on the distance from the log's head, with temperature/cm gradients gradually decreasing by steps of 0.01 degree. Along the median circumference of the logs, the temperature increased logarithmically with the treatment length, and linearly with depth.

More precisely, in a longitudinal direction the temperature measured at 30 mm depth after 120 min of steaming varied from 96.1°C to 69.3°C (average temperatures) moving from the end of the log to its median part (100 cm) according to the equation y=87.09517-44.1918 ln (ln x) ($R^2$=0.94; $P$=0.0001; Fig. 1). In 120 min, at 100 cm from the logs' head a temperature of 60°C, which devitalised the parasite *in vitro* in 15 min, was always exceeded. In a radial direction, temperatures measured at 3 cm depth always increased. The phenomenon varied logarithmically, and at 3 cm depth was expressed by the equation y= -18,2253 + 17,80246 ln x ($R^2$=0.95; $P$=0.001; Fig. 2), according to which a temperature of 60°C is reached in at least 110 min (lower confidence limit). The average moisture content of the logs before and after the trial was 114.4 and 102.9%, respectively.

The results and statistical analyses therefore demonstrated that under the experimental conditions, at a 3 cm depth in the median section of the log in a moisture saturated environment, 60°C was reached with a steam-flow treatment at 99.5°C for 120 min, to which at least 15 min must be added for parasite devitalisation.

**Trials on wood logs stem-treated on lorries**

The test performed on logs loaded on a lorry demonstrated that, on 350–450 cm long, 60–80 cm diam. logs, the parasite previously present in all the wood samples before the trial was 100% devitalised by steaming at 99.5°C for 180 min.

Although the minimum treatment times must be better standardised depending mainly on the log sizes, their rhytidome thickness, and both log and environment temperatures (Giordano, 1983), we can reasonably assume that with logs up to 3.5 m long, 60 cm in median diameter, with cracked rhytidome, and a log and air temperature of more than 22°C, successful steam treatment on a lorry must have a minimum duration of 180 min. The

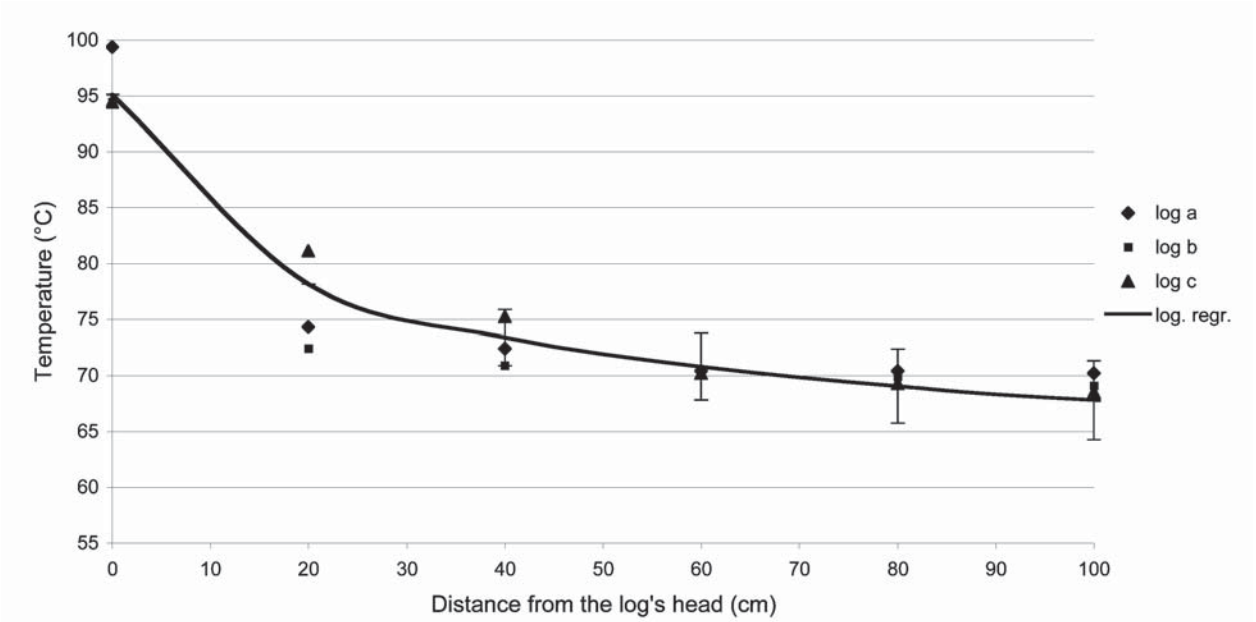


Fig. 1. Temperature variation with the distance from the head of a log after 120 min at 30 mm depth. Confidence intervals refer to the equation y=87.09517–44.1918 ln (ln x) ($R^2$=0.94).

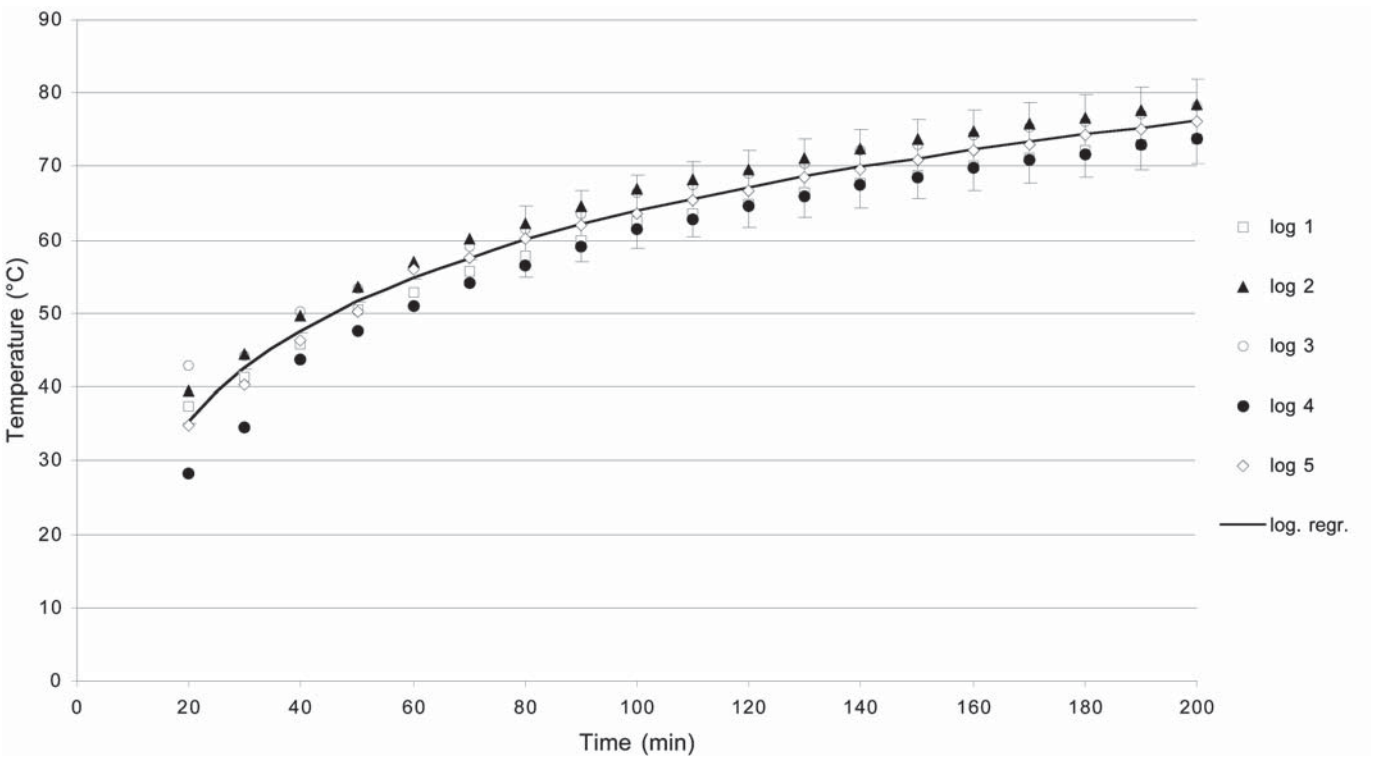


Fig. 2. Temperature variation with time at 100 cm from the head of a log and at 30 mm depth. Confidence intervals refer to the equation y=-18,2253 + 17,80246 ln x ($R^2$=0.95).

duration must be longer both with higher biomass to heat and in colder weather.

On this basis, the following technical-operative protocol is proposed for hygrothermal treatment directly on the lorry.

1. All the material and methods, and all personnel involved, must comply with the safety regulations in force.
2. The site chosen for the treatment must have a water intake of at least 1 $m^3 h^{-1}$ capacity to supply the boiler.
3. In the parking place, a heat-resistant PVC tarpaulin should be laid on the ground of a size that permits the condensed water percolating during the treatment to be collected, which must be disposed of and/or used according to the regulations in force.
4. Depending on the amount and size of the materials to be treated, boilers must supply 500–1000 kg $h^{-1}$ of steam. It is necessary to allow for heat dispersal that will occur in the tube between the boiler and the lorry (ca. 20 m). The boiler must therefore supply steam flowing into the lorry body at a temperature of above 98°C.
5. The body of the lorry on which the logs are loaded must have openings to allow both steam and percolated water to escape.
6. If contact between logs obstructs the normal circulation of the steam, especially in the central part of the pile, woody or heat-resistant plastic spacers must be used.
7. A flexible PVC hydrant hose (6" diam. circa) with the internal rubber sheath removed must be connected to the boiler and placed around the load.
8. One or more probes must be connected to a digital thermometer in order to measure the temperature in any critical zones inside the steam chamber.
9. The lorry body must be closed with heat-resistant PVC tarpaulins (e.g. Plyvil SL, Plyvil Nk or Teflon, more tear-resistant).
10. The tarpaulin must be fixed to the edges of the lorry-body using wooden boards held by clamps spaced approx. 1.5–2 m apart in order to reduce steam escaping.
11. The steam chamber thus formed must allow a stationary state to be reached in a short time, after which the steam flow entering is compensated by the one leaving through the escape routes. If the body has a floor without openings, the tarpaulin must be left slightly open at some points by slackening the closure of one clamp on either side.
12. After the boiler is switched on, at least 3 hours must pass for the material to be considered cured of *Cryphonectria parasitica*.
13. After the boiler is switched off all the equipment must be removed before the material is transported. It is advisable to leave the tarpaulin on the lorry for a sufficient time to limit any risk of wood splitting due to excessive temperature excursion.
14. Treatment can be avoided when logs are destined for shearing, which involves longer steaming times, and if routes are taken along roads that do not pass by any forests or nurseries where *C. parasitica* hosts grow.

## Conclusions

The procedure reported, associated with the usual and necessary official controls at EU borders, when performed as described, devitalises the pathogen in logs directly on the lorry, preserving the commodity characteristics of the wood.

Although the import of chestnut logs with rhytidome is forbidden only in explicit European *protected zones* (European Council, 2004), as the actual EU rules allow member Countries to issue further protecting measures, the suggested procedure can represent a widely exploitable tool against the introduction of foreign *C. parasitica* virulent strains that could generate new vegetative compatibility groups, isolated by the local ones and therefore able to reduce the hypovirulence spread in biological control plans (Causin *et al*., 1995).

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