Impact of ‘brown rot’ caused by *Gnomoniopsis castanea* on chestnut fruits during the post-harvest process: critical phases and proposed solutions

Carmen Morales-Rodriguez, a Giorgia Bastianelli, a Romina Caccia, a Giacomo Bedini, a Riccardo Massantini, a Roberto Moscetti, a Thomas Thomidis b and Andrea Vannini a, b *

Abstract

BACKGROUND: The brown rot fungus, *Gnomoniopsis castanea*, is the main organism responsible for the outbreak of chestnut postharvest decay that is threatening the sustainability of the chestnut market in Europe. Currently, no specific strategy is available to mitigate the impact and remediate the high losses of fruits in postharvest storage. In the present study, the different phases of chestnut handling in a standard facility plant were analyzed by evaluating the amount of fruit rot and infection by *G. castanea* at each phase.

RESULTS: The warm bath (48 °C) was identified as the critical phase, requiring strict parametrization to effectively inactivate *G. castanea* in fruits. Laboratory tests indicated that maintaining fruits at 50 °C for a maximum of 45 min provided optimal conditions to completely inactivate *G. castanea* inoculum during postharvest handling. However, the warm bath at 50 °C and over was not effective in inactivating the complex of fungal taxa responsible for contamination and development of molds. Higher temperatures and extended treatment times caused significant losses in fruit quality, as indicated by taste panel evaluation. Upscaling of postharvest facilities is discussed and critically evaluated.

CONCLUSION: The warm bath (50 °C for 45 min) is effective in completely inactivating *G. castanea* in fruits but did not reduce the impacts of the complex of molds responsible for external contamination and mycotoxin production.

© 2021 The Authors. *Journal of The Science of Food and Agriculture* published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Supporting information may be found in the online version of this article.

Keywords: Castanea sativa; internal decay; sterilization; fungal contamination; sensory qualification

INTRODUCTION

Before the outbreak of the brown rot fungus *Gnomoniopsis castanea*, G. Tamietti (syn. *Gnomoniopsis smithogilvyi* L.A. Shuttleworth, E.C.Y. Liew 26 & D.I. Guest),1,2 fruit spoilage caused by insects, fungi, bacteria, and abiotic factors was considered to be one of the problems of the sweet chestnut industry and market in different production regions worldwide, but generally affecting a low percentage of fruits in storage. As a consequence, various nut-treatment techniques were developed. The most common treatments are water curing (*curatura* in Italian) and thermo-hydrotherapy (warm bath), developed in Italy during the 1930s.3 The *curatura*, known in the past as ‘novena’, because fruits were usually kept in water for 9 days,4 is a classical method used for its proven ability to control insect infestation, killing the larvae of pests (mainly *Curculio* spp. and *Cydia* spp.) and molds without influencing nut quality.5–7 In contrast, sterilization consists of submerging the nuts in steel bins containing a warm water at a temperature recommended to be in the range of 48–52 °C for 35–
This new disease was named brown rot for its typical symptoms including *Acrosperma mirabilis*, *Alternaria* spp., *Aspergillus* spp., *Botrytis cinerea*, *Sclerotinia pseudotuberosa*, *Colletotrichum acutatum*, *Fusarium* spp., *Mucor* spp., *Penicillium* spp., *Phoma castanea*, *Phomopsis endogena*, *Phomopsis viterbensis*, *Rhizopus* spp., *Trichoderma* spp., and *Trichothecium roseum*.2,13–19

Postharvest problems in sweet chestnut changed in the first decade of the 21st century, when European and Australian chestnut growers first noticed a severe increase in internal rot in the fruits, locally affecting up to 80% of production in particular areas.2,20-22 This new disease was named brown rot for its typical symptomatology and was demonstrated to be caused by *G. castanea*, a fungus commonly found as an endophyte in most chestnut tissues and known to cause a range of different symptoms in the genus *Castanea* and other tree species.19 The severe impact of the pathogen in the last decade was associated with a massive presence of inoculum in the environment boosted by climate change,23 and in synergy with infestation by the Chinese gall wasp *Dryocosmus kuriphilus* Yasumatsu.24-26 Chestnut rot results in browning and necrosis of the endosperm and embryo.1 The symptoms are mainly expressed postharvest, although rotting fruits can be found in burrs on the tree before harvest.20 Notably, fruits that appear healthy have latent infections of the pathogen that, in conditions of optimal temperature and presence of water, rapidly colonize the endosperm in postharvest conditions. Quantifying the impact suggests that incidence may be as high as 93.5% in north-west *Europe,2,23 72% in Australia,22 and 21% in Switzerland,27 the disease was more recently recorded in North America28 and Chile (Vannini, unpublished).

The sudden outbreak of chestnut rot exacerbated the problems of fruit spoilage, putting the sustainability of the chestnut market at serious risk in different regions of Europe: ‘business-as-usual’ fruit-handling methods were not able to remediate the strong impact of the disease postharvest. Thus, efforts are required to analyze the commonly employed postharvest handling chain, determining the critical phases that exacerbate the problem, and those that may best contribute to a reduction in brown rot incidence.

The aims of the present study were (i) to evaluate the impact of *G. castanea* and associated brown-rot symptoms at each phase of handling under standard conditions, and to highlight critical phases in postharvest handling; and (ii) to determine the optimum parameters for sterilization to assure complete and durable inactivation of *G. castanea* inoculum, without affecting the organoleptic characteristics of the fruit.

**MATERIALS AND METHODS**

This work was carried out during the 2018 harvest season in one of the largest chestnut-producing districts in Central Italy, the Monti Cimini area (province of Viterbo, Italy), hitherto characterized by highly productive managed orchards and coppices. The chestnut fruits analyzed in this study were harvested during the 2018 production season and were provided by the Mastrogregori Ltd processing facility (www.mastrogregori.it; Valverio, Italy). Chestnut fruits at this facility are passed through the following phases: (P1) delivery to the plant when a rough estimation of overall damage is carried out by visual assessment of 0.1% per batch with a minimum of 1 kg; a first grading is carried out, and the product is separated from impurities by forced air; (P2) *curatura* when the product is immersed in tap water (10–15 °C) for 72–96 h in large bins (ratio fruits: water = 2:1 v/v); (P3) soaking phase, where the product is rapidly immersed in tap water in a large bin – floating fruits and debris are removed; (P4) the sterilization phase (warm bath) at a temperature of 48 °C for 35–40 min in continuous flow (ratio fruits: water = 1:1 v/v) followed by a cooling phase in tap water (12–17 min) and drying with forced air. The equipment can handle up to 5000 kg h–1 of chestnuts. The water in the warm bath is changed daily and the tank is cleaned. The water in the cooling tank is changed continuously to maintain a low temperature (15 °C); (P5) grading and manual selection before storage in large refrigeration cells at 0 ± 2 °C and 90% ± 1% RH.

**Impact and presence of *Gnomoniopsis castanea* and total fungal community during chestnut handling phases in a chestnut warehouse**

*Evaluation of the presence of G. castanea in chestnut fruits*

Three fruit samples (500 ± 30 g; ca. 30 fruits per sample) were collected randomly at the end of phases 1 to 5; half of the sample was analyzed at time 0 (T0) and the other half was stored for 30 days (T30) at 0 °C. Fruits were opened, the presence of ‘brown rot’ was recorded, and symptomatic fruits were analyzed to check for the presence of *G. castanea*. Isolation in pure culture was carried out as follows: after shell removal, each kernel was split in half with a sterilized razor blade. Five fragments of each sterilized fruit were placed onto Petri dishes containing potato dextrose agar (PDA) (Oxoid, Basingstoke, UK, 39 g L–1) amended with streptomycin sulphate (0.06 g L–1) (PDAs) and incubated at 20 ± 1 °C. After 7 days of incubation, cultures were scored for the presence of *G. castanea* colonies. Cultures were re-scored after 30 days to confirm the absence of growth on fragments that were scored negative at day 7. Identification was based on the morphology of the colony and reproductive structures and was ultimately confirmed by molecular barcoding using the protocol reported by Morales-Rodriguez et al.20 DNA was extracted from fresh mycelium grown in PDA with the NucleoSpin Plant II mini kit (Mackery Nagel, Düren, Germany) following the manufacturers’ instructions. The nuclear Internal Transcribed Spacer (ITS) of rDNA was amplified and sequenced from a subset of each sample using the primers ITS4 and ITS5F.31 Amplicons were purified with NucleoSpin Gel and PCR Clean-up (Mackery Nagel, Düren, Germany). Sequencing reactions were performed by Eurofins Genomics Laboratory (Ebersberg, Germany). Forward and reverse sequences were assembled and edited using BioEdit (Ibis Bioscience, Carlsbad, CA, USA) and compared to accessions in the National Center for Biotechnology Information (NCBI) database. Data were expressed based on the percentage of rotted fruits and the percentage of fruit where *G. castanea* was isolated. The experiment was repeated twice.

*Evaluation of the total fungal community in water during the sterilization and cooling phase*

One-and-a-half liters of water were collected from each of the sterilization tank (48 °C) and the cooling tank in phase 4. Serial dilutions were prepared using the protocol described by Hirte;22 45 min followed by rapid cooling in tap water (c.a. 15 min).5,8,9 This method was proposed to kill insect larvae in a shorter time than the *curatura*, although it could also reduce, but not eliminate, the mycoflora contaminating the fruits.5,8,9 More recent studies introduced the use of hot air-assisted radiofrequency treatments as a physical method to reduce the rate of mold contamination of chestnut fruits in storage, specifically targeting *Penicillium* spp.10 Studies were published employing a combination of biological and physical methods,11 or the use of a range of chemical treatments12 targeting contaminating microorganisms. These methods reduced contamination by a range of fungal taxa associated with the spoilage and rot of the product in postharvest, including *Acremonium pullulans*, *Alternaria* spp., *Aspergillus* spp., *Botrytis cinerea*, *Sclerotinia pseudothuberosa*, *Colletotrichum acutatum*, *Fusarium* spp., *Mucor* spp., *Penicillium* spp., *Phoma castanea*, *Phomopsis endogena*, *Phomopsis viterbensis*, *Rhizopus* spp., *Trichoderma* spp., and *Trichothecium roseum*.5,13–19
and T30 (unpaired t-test; P < 0.0001).

500 μL of each dilution was plated on PDA; ten dishes per dilution were prepared and the experiment was repeated twice. Fungal colony-forming units (CFU) were counted after 72 h of incubation at 20 ± 2 °C. Fungal morphotypes were transferred on fresh PDA, incubated for 7 days at 20 ± 2 °C and identified by sequencing the ITS region, as described in the previous subsection.

**Laboratory trials to evaluate the efficacy of warm bath treatment on the survival of G. castanea in chestnut fruits**

To investigate the impact of the warm bath treatment on the survival of G. castanea inoculum, the experimental design included temperature and time as factors. Thus, three temperatures (45, 50, and 54 °C) plus control (20 °C), and three times of exposure (30, 45, and 60 min) were tested, for a total of 12 treatments. A circulating water bath was used (TSCR35, ThermoFisher Scientific, Waltham, MA, USA) with temperature stability of ±0.1 °C and temperature uniformity of ±0.05 °C. There were two replicates of 1 kg (ca. 60 fruits) per treatment. After water treatment, each sample was cooled in cold water (4 °C) for 1 h and air dried until the weight before treatment was attained. After 15 days at 0 °C in a cold room (90% ± 1% RH and airflow 12 m³ h⁻¹), the rot class from all fruits treated was assessed. The internal rot class was estimated according to a visual scale where 0 = healthy fruit; 1 = rot up to 50%; 2 = rot >50%. For each treatment, six chestnut fruits were randomly sorted from each rot class (0, 1, and 2) and five fragments of each fruit were placed onto Petri dishes containing PDA before incubation at 20 ± 2 °C. After 7 and 30 days of incubation, plates were scored for the presence of G. castanea as described above. Data were expressed as the number of G. castanea positive fragments.

Furthermore, 10 mL of water used on each treatment was analyzed for the presence of G. castanea by serial dilutions, as described above.

**Sensory evaluation of chestnut fruits after warm bath treatment: laboratory trials**

A sensory evaluation was carried out to evaluate changes in the main organoleptic traits of fruit after hot-water treatment. Four temperature treatments (50, 54, 58, and 60 °C) effective in inactivating G. castanea inoculum, three times of exposure (30, 45, 60 min) and two storage times (no storage, 2 weeks of storage at 0 °C in cold room) were combined in a randomized block design to obtain 48 different treatments (1 kg of chestnuts per treatment). The quantitative descriptive analysis (QDA) method was used by a group of ten trained panelists as an analytical-descriptive method. The members of the panel were similar in experience, and training background. A sensory evaluation environment was set up with white booths and plastic containers to hold each test sample and water. The containers held five fruits and the tasters were invited to eat as many fruits as they wished from each treatment and then to fill in the evaluation. The samples were evaluated from left to right and the order was randomized in each booth. The sensory evaluation took place in a single session and was recorded manually using report forms especially prepared for the test.

Table 1. Qualitative descriptors used in for sensory analysis

| Descriptors         | 1               | 2               | 3               | 4               |
|---------------------|-----------------|-----------------|-----------------|-----------------|
| Crispness           | normal          | slightly altered| altered         | absent          |
| Bitterness          | absent          | tolerable       | bitter          | very bitter     |
| Sweetness           | normal          | Slightly altered| sweet           | very sweet      |
| Shell color         | normal          | slightly altered| altered         | very altered    |
| Pulp color          | normal          | slightly altered| altered         | very altered    |

Figure 1. Percentage of brown rot (± SEM) (A) and percentage of isolation of G. castanea from rotten fruits (± SEM) of Castanea sativa (B) in the five defined phases of postharvest; at time 0 (T0; black bars) and after 30 days of storage at 0 °C (T30, grey bars). The same lower case letters indicate no significant differences among phases (Tukey’s HSD post-hoc test). The same upper case letters indicate no significant differences at each phase between T0 and T30 (unpaired t-test; P < 0.001).

Figure 2. Fungal colony-forming units (CFU mL⁻¹ ± SEM) in 50 °C sterilization water (black bar) and cooling water (gray bar). Values followed by different letters differ significantly (unpaired t-test, P < 0.0001).
Impact of ‘brown rot’ caused by Gnomoniopsis castanea on chestnuts

Four sensory attributes considered important for consumer acceptance and potentially affected by warm bath treatment were chosen for evaluation: crispness, bitterness, shell color, and pulp color. Sweetness was also considered as the indication of a higher risk of perishability due to the release of free sugar as a substrate for contamination by saprotrophic microorganisms. Qualitative variables of the descriptors were transformed into quantitative variables using a numerical four-degree scale (Table 1). The evaluation was carried out using untreated chestnuts as controls, for which all descriptors were scored 1.

Table 2. General linear model (GLM) for G. castanea isolation with water temperature, time of treatment, and rot class, as categorical factors

| Source                        | Sum of squares | df | Mean sum of squares | F-test | P-value |
|-------------------------------|----------------|----|---------------------|--------|---------|
| Temperature                   | 100.463        | 1  | 100.463             | 48.05  | <0.0001 |
| Rot class                     | 75.6594        | 2  | 37.8297             | 18.09  | <0.0001 |
| Time                          | 0.366072       | 2  | 0.183036            | 0.09   | 0.9162  |
| Temperature * rot class       | 33.6612        | 2  | 16.8306             | 8.05   | <0.001  |
| Temperature * time            | 1.47767        | 2  | 0.738 837           | 0.35   | 0.7030  |
| Rot class * time              | 6.36837        | 4  | 1.59209             | 0.76   | 0.5521  |
| Temperature * rot class * time| 4.49737        | 4  | 1.12434             | 0.54   | 0.7082  |
| Residual                      | 275.982        | 132| 2.09077             |        |         |
| Total                         | 54.824         | 149|                     |        |         |

Statistical analysis

Data normality and equal variances were tested using the Kolmogorov-Smirnov and Bartlett tests, respectively. Percentages of brown rot and G. castanea isolation in the chestnut-handling phases fulfilled these assumptions and were further analyzed by a one-way ANOVA, followed by Tukey’s honest significant difference (HSD) post-hoc tests at the 95% family-wise confidence level (P < 0.05). To compare between two sets as the percentages of brown rot and G. castanea isolation at T0 and T30, and CFUs from water collected from the sterilization tank and the cooling tank in phase 4, an unpaired Student’s t-test was performed. All analyses were carried in Prism version 8.00 (GraphPad Software, San Diego, California, USA).

Data from laboratory trials to evaluate the efficacy of hot-water treatments were not normally distributed; therefore, a general linear model (GLM) was employed using Statgraphics Centurion XVI, v16.1.18 (Statgraphics.Net, Madrid, Spain). A Welch ANOVA and Games-Howell’s multiple comparison tests were applied to analyze CFUs from water samples in laboratory trials because of significant differences in Bartlett’s tests for equal variances. Analyses were carried out in Prism version 8.00.

Past version 4.0 (Paleontological Statistics Software, Oslo, Norway) was used to obtain a radar chart of the panel test data.

RESULTS

Impact and presence of Gnomoniopsis castanea and total fungal community during chestnut handling phases in a chestnut warehouse

Evaluation of the presence and impact of G. castanea in chestnut fruits from standard treatment

The one-way ANOVA showed significant differences (P < 0.001) in the percentage of rotted fruits among phases at both storage times (T0; F = 16.87; T30; F = 7.14). The percentage of fruits with rot increased during the phases of the postharvest handling: treatments carried out in phases 2 and 4 (curatura and sterilization, respectively) presented the lower percentage of rot (16%) but this increased to 25% at phase 5 (manual sorting). Tukey’s HSD post-hoc test results are shown in Fig. 1(A). After 30 days of storage, the percentages of brown rot doubled in all phases. In the case of the last phase (manual sorting) corresponding to a commercially ready product, the percentage increased from 25% to 54%. An unpaired t-test between T0 and T30 showed significant differences at each phase, with the percentage of brown rot always higher after 30 days (Fig. 1(A)). In the same way, for the isolation of G. castanea, a one-way ANOVA showed a significant reduction (P < 0.001) in the percentage of isolation during the postharvest handling phases analyzed at both times.
Treatments at 50 and 54 °C resulted in the total and permanent inactivation of \textit{G. castanea} after storage at P4 (F = 4.30; \(P < 0.001\)). Rot class was a significant factor (F = 16.24; \(P < 0.001\)), whereas time and the interaction rot class \times time interaction were not (F = 0.03 and F = 0.52 respectively; \(P > 0.05\)). Tukey’s HSD post-hoc test showed significant differences between rot class 0 (healthy fruits) and rot classes 1 and 2 (Fig. 3(A)). At 45 °C, the GLM was also significant (F = 2.20; \(P < 0.05\)). The rot class was significant (F = 2.21; \(P < 0.005\)), but time and the interaction rot class \times time were not (F = 1.03 and F = 1.21 respectively; \(P > 0.05\)). Tukey’s HSD post-hoc test showed significant differences between the rot class 0 and classes 1 and 2 (Fig. 3(B)).

A presence of fungal CFUs was detected in all warm bath treatments although in decreasing numbers from 45 °C to 54 °C (Fig. 4). For each time (30, 45, 60 min) significant differences were found between temperatures: 30 min (W = 13.89; \(P = 0.001\)); 45 min (\(W = 13.89; P = 0.0018\)), and 60 min (\(W = 72.04; P < 0.0001\)).

A total of seven morphotypes were identified, three of which (\textit{Penicillium echinulatum}, \textit{Galactomyces geotrichum}, and members of the Mucoraceae) represented 79.3, 13.5, and 6.9% of the total CFU. \textit{Gnomoniopsis castanea} was not detected from sterilization water in any temperature, time, or rot class.

**Sensory evaluation of chestnut**

The values for each descriptor are shown in a radar chart at T0 (a) and T15 (b) (Fig. 5). At T0, differences compared with the control (fruits in water at 20 °C; T0, descriptors reference value = 1) were evident but within 40% deviation, except for the treatments at 58 °C for 45 and 60 min, where differences were over 50%. At T15, the fruits treated at 50 °C for 30 and 45 min deviated from the controls (fruits in water at 20 °C; T15, descriptors reference value = 1) by 4% and 6% respectively, whereas deviations were much higher for the other treatments, ranging from 30% to 154%. The results of the sensory evaluation of chestnut fruits are reported as a single descriptor and the total score for each fruits that were analyzed, regardless of the storage time and rot class. Thus, these two treatments were not considered in the following statistical analysis. The GLM, with \textit{G. castanea} isolation as dependent variable and temperature, time (30, 45, 60 min), and rot class (0, 1, 2) as categorical factors, was significant (F = 9.56; \(P < 0.0001\)) (Table 2).

However, because of the significant temperature \times rot class interaction (\(P = 0.0005\)), GLMs were performed for each temperature (20 and 45 °C) with rot class as categorical factors. At 20 °C, the GLM was significant (F = 4.30; \(P < 0.001\)). Rot class was a significant factor (F = 16.24; \(P < 0.001\)); whereas time and the interaction rot class \times time interaction were not (F = 0.03 and F = 0.52 respectively; \(P > 0.05\)). Tukey’s HSD post-hoc test showed significant differences between rot class 0 (healthy fruits) and rot classes 1 and 2 (Fig. 3(A)). At 45 °C, the GLM was also significant (F = 2.20; \(P < 0.05\)). The rot class was significant (F = 2.21; \(P < 0.005\)), but time and the interaction rot class \times time were not (F = 1.03 and F = 1.21 respectively; \(P > 0.05\)). Tukey’s HSD post-hoc test showed significant differences between the rot class 0 and classes 1 and 2 (Fig. 3(B)).

A presence of fungal CFUs was detected in all warm bath treatments although in decreasing numbers from 45 °C to 54 °C (Fig. 4). For each time (30, 45, 60 min) significant differences were found between temperatures: 30 min (W = 13.89; \(P = 0.001\)); 45 min (\(W = 13.89; P = 0.0018\)), and 60 min (\(W = 72.04; P < 0.0001\)).

A total of seven morphotypes were identified, three of which (\textit{Penicillium echinulatum}, \textit{Galactomyces geotrichum}, and members of the Mucoraceae) represented 79.3, 13.5, and 6.9% of the total CFU. \textit{Gnomoniopsis castanea} was not detected from sterilization water in any temperature, time, or rot class.

**Sensory evaluation of chestnut**

The values for each descriptor are shown in a radar chart at T0 (a) and T15 (b) (Fig. 5). At T0, differences compared with the control (fruits in water at 20 °C; T0, descriptors reference value = 1) were evident but within 40% deviation, except for the treatments at 58 °C for 45 and 60 min, where differences were over 50%. At T15, the fruits treated at 50 °C for 30 and 45 min deviated from the controls (fruits in water at 20 °C; T15, descriptors reference value = 1) by 4% and 6% respectively, whereas deviations were much higher for the other treatments, ranging from 30% to 154%. The results of the sensory evaluation of chestnut fruits are reported as a single descriptor and the total score for each

![Figure 5](https://example.com/figure5.png)

**Figure 5.** Radar chart of the sensory evaluation of chestnut fruits for each descriptor at T0 (no storage; A) and T15 (after 15 days of storage at 0 °C; B).
The present study suggested that the some rotten fruits enter the plant and pass through all the post-destructive mycological analyses of fruit samples; nevertheless, sweet chestnuts in the delivery phase (P1). On delivery, there is the handling plant, active inoculum is mostly introduced with tests suggested that a warm bath at 50°C reduce the impact of

Several previous publications reported work aiming to control the complex communities of organisms other than G. castanea responsible for fruit spoilage. The fungistatic effect resulting from P4 continued into the gradating and manual selection phase. This phase did not significantly affect both the percentage of rotten fruits or the frequency of isolation of G. castanea, although it did not result in total inactivation of inoculum in the running conditions at the handling plant (48°C; 40 min). The effect was more fungistatic than fungicidal because after 30 days storage isolation frequency returned to the values obtained immediately after phase 1. As also reported by Jermini et al., the warm bath (45–48°C) did not suppress the complexity of mold species present in the chestnuts, some of which also produce mycotoxins; the molds were easily recovered from both the warm bath and the cold bath of the cooling phase. These taxa were the same species or co-generic with those responsible for causing molds in chestnuts, including Penicillium spp. Several previous publications reported work aiming to develop sustainable chemical and biological treatments of chestnuts postharvest treatments to reduce fruit spoilage. The most effective techniques must be considered in the near future to control the complex communities of organisms other than G. castanea responsible for fruit spoilage.

The fungistatic effect resulting from P4 continued into the gradating and manual selection phase. This phase did not significantly affect both the percentage of rotten fruits or the frequency of isolation of G. castanea. The whole process in the facility plant did not reduce the impact of G. castanea, as demonstrated by the severe and significant increase in the percentage of rotten fruits after 30 days of storage at 0°C. The sterilization phase is the crucial stage for further work to optimize the process and thereby reduce the impact of G. castanea in chestnuts postharvest, thus increasing storability, safety, and shelf-life of the fruit. Laboratory tests suggested that a warm bath at 50°C for 30 or 45 min was sufficient to inactivate G. castanea permanently, in chestnuts regardless of the level of colonization and rot stage. Longer periods and higher temperatures were also effective, but negatively impacted the sensory characteristics of the fruits. Lower temperatures, 45°C, effectively inactivated G. castanea inoculum in fruits with rot symptoms, but when affecting less than 50% of the pulp. With greater amounts of rot, this temperature did not permanently inactivate the pathogen, regardless of the length of the treatment (30, 45, or 60 min). This point is of particular relevance in the general context of the control of brown rot. As reported above, the sterilization tanks in a modern chestnut postharvest facility process several tons of chestnuts per hour in a continuous flow. The mass of fruits enters the sterilization phase from the cold bath and the floating phase, at a temperature of approximately 15°C. To guarantee the efficacy of the warm bath treatment, the mass of fruits must first be taken to the temperature of 50°C and subsequently maintained at this temperature throughout the fruit mass for at least 30 min. Such conditions are relatively easy to be set and monitor in laboratory conditions but upscaling to the production facility makes monitoring and setting of strict parameters more difficult, depending on the time required to bring the whole mass to the correct temperature and to maintain that temperature for the proper time. It is important to maintain the treatment for sufficient time to totally inactivate the G. castanea inoculum in fruits, as a fungistatic effect would not prevent the outbreak of the disease in the storage phase. It must also be considered that a processing system working in continuous flow must synchronize the different phases to guarantee the economic sustainability of the process itself. It might be argued that an increase in the temperature would better guarantee the inactivation of the pathogen and reduce the time of the treatment. However, in the present study, it was evident that treatments at temperatures higher than 50°C for longer than 45 min irreversibly compromised the sensory quality of the fruits. The sensitivity of fruits to heat treatments strictly depends on the varieties of chestnut in addition to the parametrization of the treatment and the storage conditions. In general, treatments <42°C preserve cell integrity even if the enzymatic activity related to ripening and maturation of the fruit are altered. Temperatures >45°C are considered near the threshold of cell damage with a limited recovery ability. There are no studies available on the specific temperature and conditions causing cell damage in chestnut fruits but it is likely that at a temperature higher than 50°C, a change in pulp color, loss of tissue consistency, and increasing bitterness were indicative of the occurrence of cell damage and the consequent release and oxidation of cell components such as phenol compounds. The application of appropriate protocols to control of ‘brown rot’ in chestnut orchards would probably enable more efficient postharvest treatment. Control methods able to reduce the endophytic inoculum of G. castanea in planta and the severity of the symptoms on fruits before delivery to the handling facility would reduce the risk that a high fraction of severely rooted fruits (over 50% of the pulp) enters handling. Activities are under way in different European chestnut areas to develop protocols for the control of ‘brown rot’ in orchards and to find alternatives to compounds currently recommended for use on chestnut (https://www.sancast.it/news-pagina.asp?id=892).

The effectiveness of product sterilization may be improved by selectively removing unsound chestnut fruits from the stock entering the handling plant using non-destructive methods based on near infrared (NIR) spectroscopy or X-ray computed
tomography. In fact, recent studies on the feasibility of Fourier transform near infrared (FT-NIR) spectroscopy or visible-near infrared hyperspectral imaging in detecting ‘brown rot’ of chestnut fruits demonstrated the feasibility of the approach and the need for an upscale from prototype to commercial equipment. A molecular tool for the sensitive detection and quantification of G. castaneae in symptomatic and asymptomatic fruits has been developed to quantify the G. castaneae inoculum. Furthermore, a recent study by Vettraino et al. provided promising evidence on the use of ozone in storage conditions, specifically as a fungal static option to avoid increasing chestnut rot in stored stocks. It is clear that an integrated protocol must be developed including the most promising methodologies and technologies to be applied in postharvest handling.

CONCLUSIONS
In conclusion, the present study demonstrated that application of strict conditions at the sterilization phase of chestnut handling can definitively inactivate G. castanea in non-symptomatic and rotting fruits. Sterilization at 50 °C and over was not effective in inactivating the complex of fungal taxa responsible for contamination and development of molds, many of which produce mycotoxins. The efficacy of the warm bath strictly depends on the quality of the fruit stocks entering the handling plant and the conditions to which they are exposed before the P4 phase. An efficient strategy for the control of ‘brown rot’ must include mitigation protocols applied in the open field, followed by effective physical treatments in the handling plant.

The positive results obtained in this work, albeit preliminary, were practical to a targeted application of low-impact containment methods for G. castanea and represent a promising step forward for harvest technologies employed in chestnut handling facilities. However, further studies are needed, both at laboratory and at industrial plant level, to develop a better understanding of the critical points of fungal contamination in the chestnut treatment chain.

ACKNOWLEDGEMENTS
This study was funded by the Latium Region with the SANCAST project and the Tuscany Region with the FORECAST project. A special thanks to Mastrogriego Ltd for making possible the monitoring and analysis at the handling phases at the postharvest facility and providing the product for the laboratory analysis. Thanks to Professor Woodward for the corrections to the English. Open Access Funding provided by Universita degli Studi della Tuscia within the CRUI-CARE Agreement. [Correction added on 19 May 2022, after first online publication: CRUI-CARE funding statement has been added.]

SUPPORTING INFORMATION
Supporting information may be found in the online version of this article.

REFERENCES
1 Croos P, Summerell B, Shivash R, Burgess T, Decock C, Dreyer L et al., Fungal planet description sheets: 107–127. Persoonia 28:138–182 (2012).
2 Visentin I, Gentile S, Valentino D, Gonthier P and Cardinale F, Gnomoniopsis castanea sp. nov. (Gnomoniaceae, Diaporthales) as the causal agent of nut rot in sweet chestnut. J Plant Pathol 28:411–419 (2012).
3 Voglino P, Il servizio di controllo fitopatologico sulle castagne destinate agli Stati Uniti d’America nella campagna 1928. Nuovi Ann. dell’Agricoltura 8:319–344 (1928).
4 Botondi R, Vailati M, Bellincontro A, Massantini R, Forniti R and Mencarelli F, Technological parameters of water curing affect post-harvest physiology and storage of marrons (Castanea sativa Mill., Marrone fiorentino). Postharvest Biol Technol 51:97–103 (2009).
5 Jermini M, Conedera M, Noccol M, Sassella A, Schärer H and Jelmini G et al., Influence of fruit treatments on perishability during cold storage of sweet chestnuts. J Sci Food Agric 86:877–885 (2006).
6 Blaiotta G, Di Capua M, Romano A, Coppola R and Aponte M, Optimization of water curing for the preservation of chestnuts (Castanea sativa Mill.) and evaluation of microbial dynamics during process. Food Microbiol 42:47–55 (2014).
7 Migliorini M, Funghini L, Mainelli C, Turchetti T, Canuti S and Zanoni B, Study of water curing for the preservation of marrons (Castanea sativa Mill., Marrone fiorentino cv). Postharvest Biol Technol 56:95–100 (2010).
8 Payne JA and Wells JM, Postharvest control of the small chestnut wee-vill in Inshell Chestnuts. J Econ Entomol 71:894–895 (1978).
9 Wells JM and Payne J, Mycoflora and market quality of chestnuts treated with hot water to control the chestnut weevil. Plant Dis 64:999–1001 (1980).
10 Hou L, Kou X, Li R and Wang S, Thermal inactivation of fungi in chestnut nuts by hot air assisted radio frequency treatments. Food Control 93:297–304 (2018).
11 Ruocco M, Lanzuise S, Lombardi N, Varlese R, Alberti A, Carpenito S, Woon S, Scala F and Lorito M, New tools to improve the shelf life of chestnut fruit during storage. In III International Symposium on Post-harvest Pathology: Using Science to Increase Food Availability 1144; 309–316 (2015).
12 Donis González I, Fulbright D, Ryser E and Guyer D, Efficacy of postharvest treatments for reduction of moulds and decay in fresh Michigan chestnuts. In I European Congress on Chestnut-Castanea 2009 866; 563–570 (2009).
13 Bounous G and Paglietta R, Il castagno da frutta. Ed: Edizione Agricole Bologna (1979).
14 Montalegre A and Gonzalez M, Fungi causing rots on Castanea sativa Mill. Fruits Simiente (Chile) (1986).
15 Ridé M and Gudin C, On the biology of some fungi parasitic on chestnuts and more particularly of P. endogena. Compte Rendu Hebdomadaire des Seances de l’Academie d’Agriculture de France 46 (1960).
16 Sieber TN, Jermini M and Conedera M, Effects of the harvest method on the infestation of chestnuts (Castanea sativa) by insects and moulds. J Phytopathol 155:497–504 (2007).
17 Vettraino AM, Paolacci A and Vannini A, Endophytism of Sclerotinia pseudotuberosa: PCR assay for specific detection in chestnut tissues. Mycol Res 109:96–102 (2005).
18 Wright W, Storage decays of domestically grown chestnuts. Plant Dis Rep 44:820–825 (1960).
19 Lione G, Danti R, Fernandez-Conradi P, Ferreira-Cardoso J, Lefort F, Marques G et al., The emerging pathogen of chestnut Gnomoniopsis castanea: the challenge posed by a versatile fungus. Eur J Plant Pathol 153:671–685 (2019).
20 Maresi G, Longa O and Turchetti T, Brown rot on nuts of Castanea sativa Mill: an emerging disease and its causal agent. J Phytopathol 155:497–504 (2007).
21 Shuttleworth L and Guest D, The infection process of chestnut rot, an important disease caused by Gnomoniopsis smithogibri (Gnomoniaceae, Diaporthales) in Oceania and Europe. Australas Plant Pathol 46:397–405 (2017).
22 Shuttleworth L, Liew E and Guest D, Survey of the incidence of chestnut rot in South-Eastern Australia. Australas Plant Pathol 42:63–72 (2013).
23 Lione G, Giordano L, Sillo F and Gonthier P, Testing and modelling the effects of climate on the incidence of the emergent nut rot agent of chestnut Gnomoniopsis castanea. Plant Pathol 64:852–863 (2015).
24 Fernández MM, Bezos D and Diez JJ, Fungi associated with necrotic galls of Dryocosmus kuriphilus (Hymenoptera: Cynipidae) in northern Spain. Silva Fennica 52:9905 (2018).
25 Magro P, Speranza S, Stacchiotti M, Martignoni D and Paparatti B, Gnomoniopsis associated with necrosis of leaves and chestnut galls induced by Dryocosmus kuriphilus. Plant Pathol 59:1171 (2010).
26 Vannini A, Vettraino A, Martignoni D, Morales-Rodriguez C, Contarini M, Caccia R et al., Does Gnomoniopsis castanea contribute to the natural biological control of chestnut gall wasp? Fungal Biol 121:44–52 (2017).
27 Dannert FG, Broggini GA, Gessler C and Storari M, Gnomoniopsis castanea is the main agent of chestnut nut rot in Switzerland. Phytopathol Mediterr 54:199–211 (2015).
28 Sakalidis M, Medina-Mora C, Kolp M and Fulbright D, First report of Gnomoniopsis smithohiglvi causing chestnut Brown rot on chestnut fruit in Michigan. Plant Dis 103:2134–2134 (2019).
29 Vannini A, Morales-Rodríguez C, Aleandri M, Bruni N, Dalla Valle M, Mazzetto T et al., Emerging new crown symptoms on Castanea sativa (mill.): attempting to model interactions among pests and fungal pathogens. Fungal Biol 122:911–917 (2018).
30 Morales-Rodríguez C, Dalla Valle M, Aleandri M and Vannini A, Pestalotiopsis biciliata, a new leaf pathogen of Eucalyptus spp. recorded in Italy. Forest Pathol 49:12492 (2019).
31 White TJ, Bruns T, Lee S and Taylor J, Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in PCR Protocols: A Guide to Methods and Applications, Vol. 18, ed. by Innis MA, Gelfand DH, Sninsky JJ and White TJ, Academic Press, London, pp. 315–322 (1990).
32 Hirte W, The use of dilution plate method for the determination of soil microflora. 2. The qualitative demonstration of bacteria and actinomycetes. Zentralbl Bakteriol Parasitenkd Infektionskr Hyg 123:167–178 (1969).
33 Mellano MG, Bounous G and Botta R, Valutazione mediante analisi sierologica dei frutti di cultivar piemontesi di castagno. In IV Convegno Nazionale Castagno 2005, Ed. SOI; 274–277 (2005).
34 Sinesio F, Guerrero L, Romero A, Moneta E and Lombard J, Sensory evaluation of walnut: an interlaboratory study. J Food Sci Technol 7:37–47 (2001).
35 Ertan E, Erdal E, Alkan G and Algül BE, Effects of different postharvest storage methods on the quality parameters of chestnuts (Castanea sativa mill.). HortScience 50:577–581 (2015).
36 Myers RH and Montgomery DC, A tutorial on generalized linear models. J Qual Technol 29:274–291 (1997).
37 Glantz S and Slinker B, Primer of Applied Regression & Analysis of Variance. McGraw-Hill, Inc., New York (2001).
38 Donis-González IR, Guyer DE and Fulbright DW, Quantification and identification of microorganisms found on shell and kernel of fresh edible chestnuts in Michigan. J Sci Food Agric 96:4514–4522 (2016).
39 Donis González I, Fulbright D, Ryser E and Guyer D, Shell mold and kernel decay of fresh chestnuts in Michigan, In I European Congress on Chestnut-Castanea 2009 866; 353–362 (2009).
40 Paull RE and Chen NJ, Heat treatment and fruit ripening. Postharvest Biol Technol 21:21–37 (2000).
41 Moscetti R, Haff RP, Saranwong S, Monarca D, Cecchini M and Massantini R, Nondestructive detection of insect infested chestnuts based on NIR spectroscopy. Postharvest Biol Technol 87:88–94 (2014).
42 Moscetti R, Monarca D, Cecchini M, Haff RP, Contini M and Massantini R, Detection of mold-damaged chestnuts by near-infrared spectroscopy. Postharvest Biol Technol 93:83–90 (2014).
43 Donis-González IR, Guyer DE, Pease A and Fulbright DW, Relation of computerized tomography Hounsfield unit measurements and internal components of fresh chestnuts (Castanea spp.). Postharvest Biol Technol 64:74–82 (2012).
44 Bedini G, Chakravartula SSN, Bastianelli G, Caccia R, Contarin M, Morales-Rodríguez C et al., Feasibility of FT-NIR spectroscopy and Vis/NIR hyperspectral imaging for sorting unsound chestnuts. Ital J Hortus 27:3–18 (2020).
45 Turco S, Bastianelli G, Morales-Rodríguez C, Vannini A and Mazzaglia A, Development of a TaqMan qPCR assay for the detection and quantification of Gnomoniopsis castaneae in chestnut tissues. Forest Pathol 12701 (2021).
46 Vettraino AM, Bianchini L, Caradonna V, Forinitt R, Goffi V, Zambelli M et al., Ozone gas as a storage treatment to control Gnomoniopsis castanea, preserving chestnut quality. J Sci Food Agric 99:6060–6065 (2019).