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Assessment of damage potential of *Gnomoniopsis castaneae* to fruit and trees of European Chestnut (*Castanea sativa*)

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Summary. *Gnomoniopsis castaneae* has become an important pathogen of European chestnut (*Castanea sativa*), affecting fruits and colonizing shoots and branches, as well as galls caused by the oriental chestnut gall wasp (*Dryocosmus kuriphilus*). On apparently asymptomatic fruits, *G. castaneae* can colonize the endosperms, causing quality issues, especially in post-harvest. Given the alarming spread of *G. castaneae* infections in Italy and the current knowledge gaps regarding aspects of *G. castaneae* biogeography and virulence, research was addressed to: 1) evaluate occurrence of *G. castaneae* in chestnut nuts and branches in different Italian regions; 2) study occurrence and distribution of the two known *G. castaneae* haplotypes throughout Italy; and 3) evaluate their virulence on chestnut under different water regimes. Fungal isolation from representative chestnut branch and nut samples consistently yielded colonies that were morphologically consistent with *G. castaneae*, the identity of which was confirmed by analysis of ITS sequences. Analysis of *β-tubulin* sequences confirmed the presence of two distinct genetic lineages (*Gc*-haplotypes A and B). To assess pathogenicity, *G. castaneae* isolates were inoculated onto chestnuts, chestnut cuttings and 3-year-old young plants grown under two water regimes. All assessed isolates were pathogenic on chestnut, and water-stressed plants exhibited more extensive necrosis than well-watered plants when inoculated with the *Gc*-haplotype A, highlighting the influence of environmental conditions on disease expression. This study expands current knowledge on the distribution, genetic diversity, and effects of water stress on the pathogenic potential of *G. castaneae* on chestnut.

Keywords. Endophytic and pathogenic behaviours, molecular typing, pathogenicity test, water stress.

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INTRODUCTION

European chestnut (*Castanea sativa* Mill.) is a versatile forest tree species, that is strongly related to human activities in Mediterranean mountain

areas (Conedera *et al*., 2004). Over centuries, chestnut cultivation has directly contributed to food security (Gabrielli, 1994), as these trees are important food resources of rural areas. Chestnut trees enabled development of local economies based on the production and commercialization of high-quality nuts, valuable timber, and a wide range of traditional processed products (Pezzi *et al*., 2022). Beyond its socio-economic importance, chestnut orchards provide key ecosystem services, including soil protection, slope stability, and erosion mitigation in mountainous environments (Bassanelli *et al*., 2013). *Castanea sativa* is also relevant for biodiversity conservation, hosting a wide range of plant and animal species, and is an efficient carbon sink (Mattioli *et al*., 2016; Prada *et al*., 2016).

In Europe, *C. sativa* is primarily cultivated in southern countries, with Spain and Portugal leading production, followed by Italy and France (Pérez-Girón *et al*., 2020). Chestnut fruits are commercialized to many countries, because they are appreciated for their low-fat content, richness in essential nutrients such as starch, sugars and proteins, and because they are gluten-free (Suna *et al*., 2021; Rodrigues *et al*., 2022; Santos *et al*., 2022).

Chestnut fruit production has declined (Maresi *et al*., 2013; Freitas *et al*., 2021), due to neglect of chestnut groves, sometimes accompanied by replacement of chestnut with more remunerative agricultural crops, and abandonment in mountainous regions as rural populations migrated (Freitas *et al*., 2021). Additional threats to chestnut ecosystems include climate change (rising temperatures), which may alter chestnut tree phenological stages, affecting fruit quality (Freitas *et al*., 2021). Spread of well-known pests and diseases, such as Asian wasp gall, chestnut blight, ink disease, and (more recently) mosaic disease, have impacted chestnut vitality and production (Battisti *et al*., 2014; Rigling and Prospero, 2018; Marais *et al*., 2021; Pezzi *et al*., 2022; Prospero *et al*., 2023). Further detrimental impacts on post-harvest production have been associated with nut rots caused by *Phomopsis endogena*, *Ciboria batschiana*, *Colletotrichum acutatum*, *Neofusicoccum parvum* and species of *Aspergillus*, *Fusarium*, *Alternaria* and *Botrytis* (Washington *et al*., 1997; Donis-González *et al*., 2009; Visentin *et al*., 2012; Gaffuri *et al*., 2017; Nicoletti *et al*., 2021; Seddaiu *et al*., 2021).

Gnomoniopsis castaneae has become an increasing threat to chestnut fruit production, causing brown rot. This fungus was independently described in 2012 by two research teams, in Italy (Visentin *et al*., 2012) and Australia (Shuttleworth, 2012), and a comprehensive review was later published by Lione *et al*. (2019). High nut infection rates have been observed in North and South America, Asia, Australia, and in Europe, particularly Switzerland (50 to 91% infection) and Italy (20 to 94%) (Lema *et al*., 2023). Origin of the fugus remains unknown, and its biology is still not fully understood (Dobry and Campbell, 2023). The life cycle of *G. castaneae* includes sexual and asexual phases. During winter, the fungus survives as mycelium and propagules in leaf litter, and galls caused by *Dryocosmus kuriphilus* in the previous chestnut growing season. In spring, ascospores released from perithecia are dispersed by wind and insects, infecting host plant female flowers, leaves and branches (Lema *et al*., 2023). During flowering, and fruit development and maturation, the fungus invades chestnut kernels, causing endosperm and embryo necrosis resulting in internal nut decay, while outer shells remain visually unaffected (Lema et al., 2023). Asexual reproduction of the pathogen, involving conidial differentiation, may occur inside infected nuts, on *D. kuriphilus* galls, or in infected flower buds, leaves or branches (Maresi *et al*., 2013; Dobry and Campbell, 2023; Topalidou *et al*., 2024).

Gnomoniopsis castaneae can be a latent pathogen able to persist endophytically within the hosts, so it is frequently isolated from apparently healthy chestnut plant woody tissues and fruits (Ugolini *et al*., 2014; Lema *et al*., 2023). Generally, its pathogenicity is associated with high levels of fruit colonization, causing brown rot, but the fungus can also induce cankers in host stem and shoots (Dar and Ray, 2015; Dobry and Campbell, 2023). Nevertheless, the biological mechanisms regulating the transition from endophytic colonization to pathogenic behaviour have not been fully elucidated (Maresi *et al*., 2013; Lione *et al*., 2016; Pasche *et al*., 2016; Shuttleworth and Guest, 2017).

The host range of *G. castaneae* is not restricted to chestnut species. The fungus has been recorded on oak (*Quercus cerris* L., *Quercus ilex* L.), pine (*Pinus pinaster* Aiton), hazelnut (*Corylus avellana* L.), and ash (*Fraxinus ornus* L.) (Visentin *et al*., 2012; Linaldeddu *et al*., 2016; EPPO, 2017; Lione *et al*., 2019; Dobry and Campbell, 2023), where it is related to cankers, fruit rot and leaf necrosis of these hosts.

Given the severe damage caused by *G. castaneae* in many countries, and the current knowledge gaps regarding some aspects of its biogeography and virulence, the present study was conducted to: 1) evaluate the occurrence of *G. castaneae* in chestnut nuts and branches across different Italian regions; 2) ascertain distributions of the two *G. castaneae* haplotypes in Italy; and 3) assess their ability to induce canker lesions on artificially inoculated chestnut stems under water-stress conditions.

MATERIAL AND METHODS

Field surveys, sampling and isolation from cankered branches

In summer 2017, the health status of chestnut trees was monitored in two orchards located in Sardinia (Aritzo: 39.950563, 9.196157) and a coppice in Veneto (Torreglia: 45.319230, 11.711188). The chestnut trees (approx. 40–50 years old) were inspected for the presence of necrosis on Asian wasp galls and for cankers on branches. A total of 20 shoots per site showing cankers, different on the colour and appearance from the ones caused by *C. parasitica*, were randomly chosen for diagnostic analysis and fungal isolations. Disease incidence (D) was calculated as the percentage of affected chestnut trees out of the total number of chestnut trees along two linear (50 m long) transects per site. Branch samples were initially disinfected with 70% ethanol for 30 s, and then used for isolation by aseptically taking ten small tissue fragments (each 5 × 5 mm) from margins of the necrotic lesions of inner bark, after removal of outer bark with a sterile scalpel. All tissue fragments were placed onto 90 mm diam. Petri dishes containing Potato Dextrose Agar (PDA, 39 g L^{-1}, DIFCO) and incubated at 25°C for 7 d in the dark. Hyphal tips from emerging colonies were then sub-cultured onto PDA in 60 mm diam. Petri dishes and then incubated at 25°C in the dark.

Nut sources and brown rot assessments

As part of the Chestnut Fruit Exhibition (Ascoli Piceno, Italy) on November 11th, 2023, the Department of Agricultural, Food and Environmental Sciences (D3A) at UNIVPM received 117 chestnut fruit samples from more than 100 chestnut varieties. Some of the trees were cultivated and preserved at the Chestnut Biodiversity Repository Fields located in Piedmont, Tuscany, Emilia-Romagna and Campania, and others were from commercial orchards in Lombardy, Trentino-Alto Adige, Marche and Sardinia (Table S1). The fruit samples (each 300 to 400 g) were collected in mid-October 2023 from the different locations. They were immediately sent to UNIVPM (Ancona, Italy), where they were stored in a cold room at 2 to 4°C under high humidity conditions until the Chestnut Fruit Exhibition, and then kept at room temperature (23°C) for 20 d prior to analyses.

Depending on nut availability for each of the 117 samples, ten to 20 chestnuts were analyzed to record morphological characteristics of the episperms (colour and texture) and, after longitudinal dissection, any signs of endosperm alterations. Green mold, and black, pink or brown rots, were recorded, along with insect larvae feeding within the nut endosperms. A more detailed assessment was conducted for chestnut brown rot, focusing on disease incidence, severity and McKinney Index.

Disease incidence (D) was calculated as the percentage of affected chestnuts (out of 20 assessed per sample).

Severity (S) was assessed by classifying each 20 chestnut sample using an empirical scale (Figure 1) (Gonzalez *et al.*, 2016 with modifications), and was calculated using the following formula:

$$S = (c \times f) / n$$

where: S = severity; c = severity class (0 to 5); f = frequency; and n = number of symptomatic chestnuts.

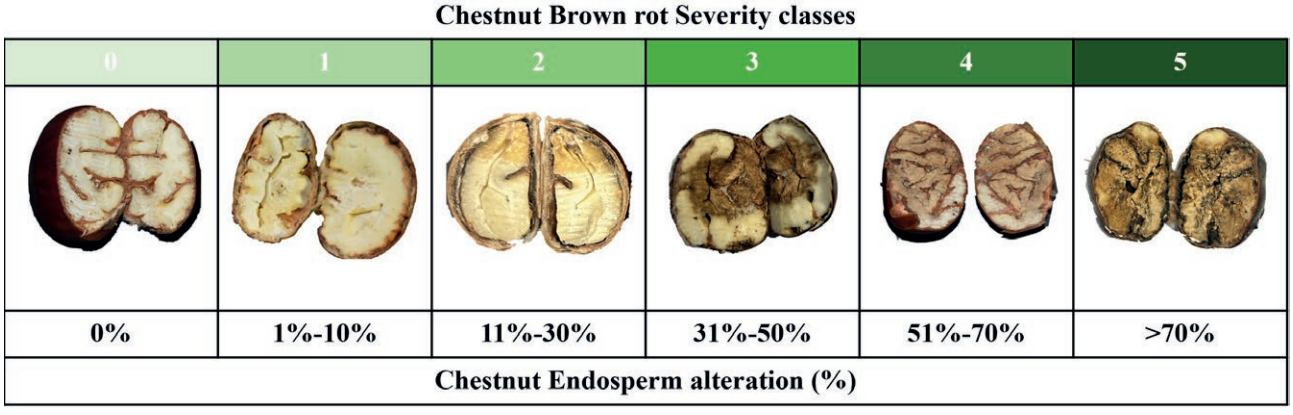

Figure 1. Chestnut brown rot severity scores, according to the percentage of endosperm alterations, as used in the present study. Six severity classes were: 0 = no disease; 1 = up to 10% diseased tissue; 2 = 11 to 30%; 3 = 31 to 50%; 4 = 51 to 70%; 5 = more than 70% endosperm diseased.

Considering both Incidence (D) and Severity (S), the McKinney Index (MI) was then calculated using the formula (McKinney, 1923; Possamai *et al*., 2023):

$$MI\ (\%) = (\Sigma(n_i \times v_i) / N \times V) \times 100$$

where: MI = McKinney Index; n_i = number of symptomatic fruits in severity class; v_i = severity class (0 to 5); N = total number of chestnuts observed; and V = maximum value of severity class.

Isolations from nut samples and morphological identification of isolated fungi

After visual assessments, microorganisms associated with tissue rot were isolated from five to ten chestnuts per sample. Fruits were surface sterilized with 90% ethanol (2 min), 2% sodium hypochlorite (2 min), then rinsed in sterile water (2 min), and then dried on sterile absorbent paper under a laminar flow hood. The fruits were then cut longitudinally with sterile blades, and asymptomatic or visibly deteriorated endosperm tissues were selected. From each fruit, five tissue pieces were placed in a Petri dish (90 mm diam.) containing 15 mL of PDA supplemented with antibiotic solution (150 mg L^{-1} ampicillin + 150 mg L^{-1} streptomycin). To assess microorganism diversity across the visual disease categories, the same procedure was repeated for 60 representative chestnuts spanning the severity classes 0 to 5. Petri plates were incubated at room temperature (23 ± 1°C) for 7 d. Resulting fungal colonies were then subcultured to obtain pure isolates. Isolate mycelium colour and structure of fruiting bodies were observed for identifications using a light microscope (Leica DM 500) at ×40 magnification, and appropriate images were captured with an ICC50W microscopic Digital USB Camera (Leica Microsystems).

DNA extraction, PCR amplification and sequencing

Among *Gnomoniopsis* isolates, 23 were randomly selected for molecular analyses, including 17 from northern Italy, two from central Italy, one from southern Italy, and three from Italian islands. Fungal DNA was extracted from each isolate using the CTAB protocol (Doyle and Doyle, 1990, with minor modifications), and was used for further molecular analyses.

Amplification reactions (total volume 25 µL) each contained 9.1 µL of MilliQ H_2O, 12.5 µL EmeraldAmp MAX PCR Master Mix 2× (Takara), 1.2 µL of each primer (10 µM), and 1 µL of DNA (50 ng). The primers used were ITS1/ITS4 (White *et al*., 1990) targeting the ITS region, and Bt2a/Bt2b (Glass and Donaldson, 1995) targeting the *β-tubulin* gene. Amplifications was carried out in a DNA iCycler (Bio-Rad), with an initial denaturation step at 95°C for 3 min, then 35 cycles, each of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 60 s, and a final elongation step of 5 min at 72 °C for both primer sets. Products were visualized on 1.5% agarose gel in 1× TAE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8), which was stained with GelRed (Biotium), and acquired using a Gel Doc system (Bio-Rad, USA). A 100 bp DNA ladder (Sigma-Adrich) was included as the size marker. Specific ITS and *β-tubulin* amplicons of the 23 representative isolates were purified and sequenced in both directions (Genewiz), then nucleotide sequences were compared with those in the NCBI database (on 16th August 2025) by nucleotide Blast Analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi). *β-tubulin* amplicon sequences were aligned with reference strains by BioEdit v. 7.2.1 (https://bioedit.software.informer.com/7.2/) to distinguish between *Gc*-haplotypes A and B, according to SNPs in the conserve region. The nucleotide sequences of ITS and *β-tubulin* regions were validated by NCBI, and were deposited in GenBank (on 3rd September 2025).

Pathogenicity tests on chestnut cuttings

Two-year-old chestnut stems (1 to 1.5 cm diam.) were harvested from ten ungrafted *C. sativa* trees without visible damage or disease symptoms. The stems, protected in plastic bags to prevent dehydration, were then stored at 4°C in a cold room for 16 h, and then cut into ~10 cm sections, which were surface-disinfected with 90% ethanol solution, and wounded (3 mm diam.) to expose the cambium. Based on previous results and molecular typing, 16 *G. castaneae* isolates were selected, including five of *Gc*-haplotype A and 11 of *Gc*-haplotype B (*sensu* Seddaiu *et al*., 2023), and two reference isolates (PD-ST30, PD-ST55) provided by the fungal collection of TESAF Department (UNIPD, Padova, Italy). Mycelium plugs from 5-d-old *G. castaneae* PDA cultures were inserted into inoculated stem wounds using sterile tools, and were then sealed with parafilm (Table 1). Negative inoculation controls received only PDA disks. The upper end of each chestnut cutting was then sealed with grafting wax, and the lower 1.2 cm portion was immersed in 3 mL of sterile water in a 50 mL tube without a cap. Cuttings were then maintained at 23±1°C for 30 d in the incubator. Length extension of necrosis on each cutting was then measured using by digital caliper (Metrica). Organism re-isolation was carried out

Table 1. Representative *Gnomoniopsis castaneae* (GC) isolates obtained from different chestnut varieties, and the respective ITS and bt nucleotide sequence registration numbers, originating from different Italian regions and molecularly characterized as Haplotype A or B based on *β-tubulin* gene typing (Seddaiu *et al*., 2021).

Gnomoniopsis isolates ID	Geographical region	Haplotype A or B	Nucleotide sequence	
			ITS	bt
GC_SanPietro	Campania	A		PX259707
GC_Barrile	Sardinia	A	PX242886	PX259715
GC_Castel del Rio-MO	Emilia Romagna	A	PX242877	PX259708
GC_Castagna di Val di Castro-SITE 1	Marche	A	PX242891	PX259719
GC_Castagna bionda di Lunano	Marche	A	PX242885	PX259714
GC_Santu Giuanni-ARI3	Sardinia	B	PX242880	PX259710
GC_Bouche de Betizac	Piedmont	B	PX242898	PX259725
GC_Marrone di Viterbo	Piedmont	B	PX242887	PX259716
GC_Precoce Migoule	Piedmont	B	PX242882	PX259712
GC_Chiusa Pesio	Piedmont	B	PX242883	PX259713
GC_Marrone scuro-2710	Lombardy	B	PX242888	PX259717
GC_Patriarca-3595	Lombardy	B	PX242889	PX259718
GC_Marrone di Perledo-3419	Lombardy	B	PX242896	PX259723
GC_Rossera-2412	Lombardy	B	PX242881	PX259711
GC_Tosca/Garfagnana-MO	Emilia Romagna	B	PX242892	PX259720
GC_Pilistella	Emilia Romagna	B	PX242899	PX259726

by removing the external layer of bark of each cutting, excising four pieces of inner bark tissues and placing the pieces onto PDA.

Pathogenicity tests on chestnut fruits

For the pathogenicity test on fruits, the local chestnut variety 'Marrone Classico di Acquasanta Terme' (from Ascoli Piceno, Marche, Italy) was selected. This variety was recorded to be poorly affected during the preliminary assessment of the present investigation. The nuts were disinfected with a superficial treatment in 90% ethanol solution for 1 min, followed by 2% sodium hypochlorite solution for 1 min), and the rinsing in sterile water, and were then left to dry on 3 mm blotting paper (Whatman) at room temperature under a laminar flow cabinet.

The 16 selected *G. castaneae* isolates (as above) were each inoculated onto four chestnut fruits, by inserting a mycelium plug (3 mm diam.), between the pericarp and perisperm of each fruit, and sealing the inoculation site with parafilm. For inoculation controls, PDA disks without mycelium were similarly added to chestnut fruits. All fruits were then incubated at 23°C (± 1°C) for 30 d, and necrotic areas were then measured as ellipses. Organism re-isolation were then carried out cutting each nut into two parts, picking out four pieces of symptomatic inner tissue and placing these onto PDA.

Pathogenicity test on chestnut seedlings under different water regimes

A greenhouse pot experiment was carried out with 3-year-old chestnut seedlings (*C. sativa*) grown in 3 L pots, that were filled with soil collected from a chestnut orchard in Montemonaco (Ascoli Piceno, Italy). All the pots were water-saturated, then two water regimes were applied: 150 mL water per week (designated WR_A) or 150 mL water per month (WR_B). The 16 *G. castaneae* isolates (as above) were then inoculated onto the seedlings. Each isolate was applied to wounds (3 mm diam.) made on the seedlings at 3, 12 and 21 cm above the soil line. After 30 d at 26°C, in greenhouse, lengths of cortex necroses were measured using a digital caliper (Metrica). Fungus re-isolations were made by removing the external layer of bark of each seedling, then excising four pieces of symptomatic inner bark tissue and placing these onto PDA in Petri plates.

Statistical analysis

For each pathogenicity test, data were tested for homogeneity of variances using Levene's test, and for normality of residuals using the Shapiro–Wilk test, prior to analyses of variance (ANOVA). When assumptions of normality and homogeneity were met, one-way ANOVAs were carried out to assess effects (at $P < 0.05$) of the different iso-

lates on sizes of necrotic tissues. If the statistical analysis showed the data were not normally distributed, the dataset was transformed using the function "Boxcox". All statistical analyses were conducted using R version 4.4.3.

RESULTS

Field symptoms and disease incidence

On monitored trees at the Sardinia and Veneto sites, chestnut blight, ink disease and Asian wasp galls were widespread. In addition, 42% of the assessed trees showed branch cankers characterized by dark necrotic bark lesions, sometimes accompanied by a reddish discolouration, mainly along the canker margins (Figure 2). The branch cankers often started from Asian wasp galls, and then progressively necrotized and advanced from the shoots towards the branches causing wilting of the distal portions. During the summer survey, affected branches were recognizable as the leaves lost their turgidity, turned from pale green to brown, and withered. Colonies of *G. castaneae* were isolated from all 60 assessed branches showing these symptoms.

Three further fungi, identified as *Dothiorella iberica* (seven isolates), *Cryphonectria parasitica* (four), and *Neofusicoccum luteum* (three) were sporadically isolated from the assessed canker samples.

Figure 2. a) Chestnut necrotic shoots; b) branch cankers starting from Asian wasp galls; c) a wilted distal portion of a tree branch; d) a canker induced by *Gnomoniopsis castaneae*.

Assessments of chestnut fruit rot

During visual inspections, the fruits generally did not show significant anomalies of pericarp colour, which are mostly due to host intraspecific variability. However, investigation of inner fruits tissues was different. After dissection, 1251 (69.04%) of 1812 chestnut samples showed endosperm diseases. More than 90% of these chestnut fruits showed no external alteration of episperm colour. From observations of the dissected fruits, the most frequent disease symptom (disease incidence; D) was brown rot (59.05%), followed by green mold (15.95%), black rot (7.06%) and pink rot (4.02%).

Chestnut brown rot was individually predominant, even when recorded with green rot (9.05%) as well as black rot (4.97%), pink rot (2.04%) and insect damage (13.74%). The different fruit rot symptoms are illustrated in Figure 3.

Focusing on the brown rot-related alteration, the average disease severity (S), was expressed as a score from 0 to 5. Most of the samples were evaluated in class 3 (31–50% of nuts), with an average severity around 3.2 (Table 2).

Combining disease incidence and severity, McKinney Index (MI) was calculated with an overall average value of approx. 42% for fruit samples of different varieties and origins (Table 2). Chestnut brown rot differently affected the varieties cultivated in the Regional Chestnut Repositories of Piedmont, Emilia-Romagna, Toscana and Campania (Table 2). Varieties most affected included 'Castagna della Madonna' (Piedmont), 'San Pietro' and 'Lucente' (Campania), 'Marrone di Loiano' (Tuscany), as well as 'Castel del Rio' and 'Bovalghe' (Emilia-Romagna), all showing high MI values (ranging from 42% to 93%). In contrast, the varieties 'Sborgà' (Emilia-Romagna), 'Salvana' (Emilia-Romagna, Tuscany), 'Marrone buono' (Tuscany), 'Ishyzuki precoce' (Piedmont), and 'Verdole' (Campania) displayed low susceptibility, with MI values ranging from 0 to 22% (Table 2).

For samples coming from chestnut orchards in Lombardy, Marche and Sardinia, some varieties had different MI values. 'Marrone scuro' (Lombardy), 'Pallante' (Marche) and 'Craeddu – CRAV' (Sardinia) were the most affected, with MI values from 33 to 100%. Conversely, several varieties had no brown rot symptoms (MI = 0%), including 'Marrone Classico di Acquasanta Terme' and 'Castagna di Val di Castro' from site 1 (MI

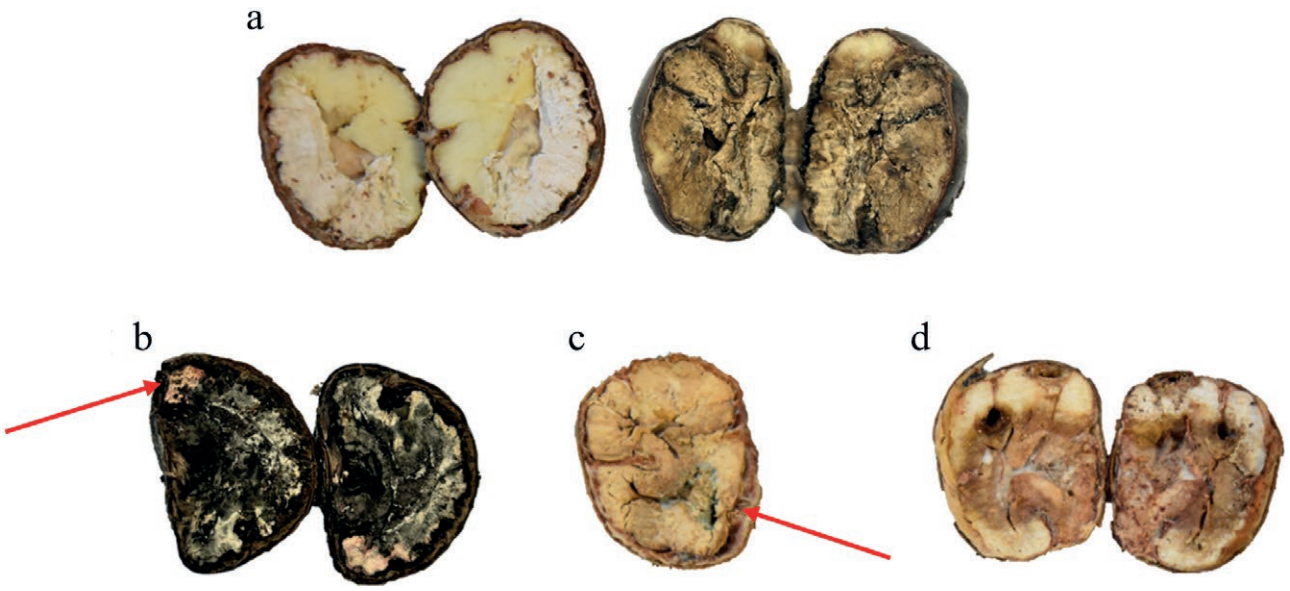

Figure 3. Symptoms recorded during visual inspections after fruit dissection. a) Chestnut brown rot; b) (arrow) black rot and pink rot; c) (arrow), green mold; and d) insect damage and brown rot.

= 0%) (Marche). Similarly, in Sardinia, the varieties 'Luccheddu – LOCG2' and 'Ildubba – ILDP' (MI = 0%) were also unaffected.

Pathogens associated with nut diseases

Most of the *in vitro* isolations from brown rot affected chestnuts from each of the 117 varieties were identified as *G. castaneae* [481 (68.52%) of 702 isolates]. In addition, *Penicillium* (4.85%), *Aspergillus* (7.41%), *Fusarium* (9.12%), *Alternaria* (6.13%) and *Trichoderma* (4.13%) were isolated at minor frequencies.

In vitro isolations from 60 chestnuts representative of the different disease severity classes (1 to 5) and asymptomatic samples (class 0), gave different proportions of *G. castaneae* isolates (Table 3). Samples of class 3 (98.3% *G. castaneae*) and 2 (95%) gave greatest proportions, followed by class 4 samples (70%), 1 (50%), and 5 (37% of samples). *Gnomoniopsis castaneae* was also isolated from 5% of asymptomatic episperms. The frequencies of *Penicillium, Trichoderma, Aspergillus, Fusarium* and *Alternaria* spp. isolations were generally greater from fruit with chestnut brown rot severity classes of 4 or 5.

Molecular identification and characterization of Gnomoniopsis castaneae

The 23 selected *G. castaneae* isolates subjected to total DNA extractions yielded from 75 and 172 ng μL^{-1} of DNA, of quality enough for further analyses (ratio 260 nm/280 nm > 1.8). All the isolates, including those with values below the quality threshold, were analyzed using the primers ITS1/ITS4 and Bt2a/Bt2b. Twenty-two of the 23 samples positively amplified for the ITS region, each showing a specific band of approx. 600 bp. For amplifications with *β-tubulin* primers, 20 of the 23 isolates gave specific bands of approx. 300 bp. Blast analysis gave 100% sequence identity when the present study *G. castaneae* isolate ITS regions were compared with that of the ex-type culture (HM142946). Blast analyses based on *β-tubulin* sequences discriminated two different *Gc*-haplotypes (A and B), according to single nucleotide polymorphisms (SNPs) in conserved position, as previously described by Seddaiu *et al.* (2023). *Gc*-haplotype A was recorded in isolates from Campania, Sardinia, Marche and Emilia Romagna, while *Gc*-haplotype B was recorded from Piedmont, Sardinia, Lombardy and Emilia-Romagna (Table 1).

Pathogenicity test on chestnut cuttings

The 16 isolates of *G. castaneae,* (five of *Gc*-haplotype A and 11 of *Gc*-haplotype B, as shown by *β-tubulin* molecular typing) induced necrotic lesions on chestnut cuttings (Figure 4A; Supplementary Table 2). From the statistical analyses, *G. castaneae* isolates of *Gc*-haplotype A were not pathogenically different ($P > 0.05$) from the *Gc*-haplotype B isolates. However, differences were found among the *G.*

Table 2. Brown rot incidence, severity and McKinney indices in chestnuts from different regions in Italy.

Chestnut variety	Region	Chestnut brown rot		
		Incidence D (%)	Severity S	McKinney Index MI (%)
Marrone di Comunanza	Marche	27.78	4.20	23.33
Insita site 1	Marche	54.55	3.33	36.36
Marrone rugoso di Acquasanta Terme	Marche	0.00	0.00	0.00
Marrone gentile di Acquasanta Terme site 1	Marche	11.11	2.00	4.44
Marrone delle Piagge	Marche	5.26	3.00	3.16
Marroncino dell'ascensione site 1	Marche	20.00	3.75	15.00
Marrone classico di Acquasanta Terme	Marche	0.00	0.00	0.00
Castagna di Val di Castro site 1	Marche	0.00	0.00	0.00
Marroncino dell'Ascensione site2	Marche	75.00	2.07	31.00
Marrone gentile di Acquasanta Terme site 2	Marche	27.78	2.40	13.33
Insita site 2	Marche	82.35	2.29	37.65
Pallante	Marche	85.00	3.24	55.00
Marrone della Sibilla di Montemonaco	Marche	25.00	3.00	15.00
Castagna di Val di Castro site 2	Marche	65.00	1.92	25.00
Castagna bionda di Lunano	Marche	10.00	2.00	4.00
Average		32.59	2.21	17.55
Marrone di Limonta – 1001	Lombardy	70.00	5.00	70.00
Marrone di Limonta – 1007	Lombardy	100.00	4.21	84.21
Agostana - 2211	Lombardy	90.00	5.00	90.00
Agostana - 2230	Lombardy	78.95	2.93	46.32
Agostana - 2233	Lombardy	95.00	4.00	76.00
Marronessa - 2338	Lombardy	94.44	4.06	76.67
Marronessa - 2339	Lombardy	100.00	4.67	93.33
Marronessa - 2340	Lombardy	93.33	4.07	76.00
Unknown - 2412	Lombardy	100.00	4.47	89.47
Rossera - 2414	Lombardy	100.00	4.15	83.00
Rossera - 2415	Lombardy	100.00	4.20	84.00
Piata - 2417	Lombardy	100.00	4.82	96.47
Enset de Piaz - 2450	Lombardy	82.35	3.79	62.35
Settembrana - 2452	Lombardy	100.00	4.53	90.59
Verdala - 2695	Lombardy	94.74	4.33	82.11
Unknown - 2896	Lombardy	100.00	4.11	82.22
Unknown - 2697	Lombardy	85.00	4.00	68.00
Marrone scuro - 2710	Lombardy	100.00	5.00	100.00
Topia - 2747	Lombardy	80.00	3.50	56.00
Barucana - 2750	Lombardy	81.25	4.00	65.00
Donegai - 3029	Lombardy	100.00	3.53	70.53
Bonela - 3039	Lombardy	95.00	2.95	56.00
Catot - 3365	Lombardy	66.67	3.40	45.33
Catot - 3366	Lombardy	100.00	3.53	70.59
Marrone - 3367	Lombardy	88.89	3.19	56.67
Catot - 3368	Lombardy	72.22	2.85	41.11
Longone - 3369	Lombardy	94.44	3.35	63.33
Marrone di Perledo - 3407	Lombardy	100.00	3.82	76.47
Marrone di Perledo - 3412	Lombardy	100.00	4.53	90.53
Marrone di Perledo - 3414	Lombardy	100.00	3.67	73.33
Marrone di Perledo - 3419	Lombardy	80.00	3.58	57.33

(Continued)

Table 2. (Continued).

Chestnut variety	Region	Chestnut brown rot		
		Incidence D (%)	Severity S	McKinney Index MI (%)
Marrone di Perledo - 3422	Lombardy	88.24	4.20	74.12
Patriarca - 3595	Lombardy	75.00	3.07	46.00
Bunela - 4712	Lombardy	100.00	4.65	93.00
Galdana - 2442	Lombardy	100.00	4.39	87.78
Average		91.59	3.99	73.54
Centa S. Nicolo'	Trentino Alto Adige	9.09	2.00	3.64
Castione	Trentino Alto Adige	55.56	3.60	40.00
Roncegno	Trentino Alto Adige	20.00	2.50	10.00
Drena	Trentino Alto Adige	22.22	3.00	13.33
Average		26.72	2.78	16.74
Loccheddu – LOCG2	Sardinia	0.00	0.00	0.00
Migheli Urru - MURG	Sardinia	66.67	2.33	31.11
Ildubba - ILDP	Sardinia	0.00	0.00	0.00
Barrile - BARV	Sardinia	55.56	2.80	31.11
Craeddu - CRAV	Sardinia	50.00	3.33	33.33
Santu Giuanni – ARI3	Sardinia	25.00	5.00	25.00
Average		32.87	2.24	20.09
Salvana	Tuscany	14.29	2.50	7.14
Marrone di Loiano	Tuscany	80.00	3.50	56.00
Marron buono di Marradi	Tuscany	26.32	1.80	9.47
Average		40.20	2.60	24.21
Verdole	Campania	35.00	3.14	22.02
Marrone di Scala	Campania	77.78	3.50	54.4
Santimango	Campania	84.21	1.44	24.21
San Pietro	Campania	100.00	4.29	85.71
Olefarella	Campania	57.89	2.91	33.68
Lucente	Campania	100.00	4.10	82.00
Tempestiva	Campania	100.00	4.05	81.00
Napoletana	Campania	100.00	3.65	73.00
Average		81.86	3.38	57.01
Marrone di Zocca - UNIBO	Emilia-Romagna	50.00	2.00	20.00
Svizzera - UNIBO	Emilia-Romagna	45.45	2.60	23.64
Pastinese - UNIBO	Emilia-Romagna	27.27	4.33	23.64
Ceppa - UNIBO	Emilia-Romagna	13.33	3.50	9.33
Sborga' site 1 - UNIBO	Emilia-Romagna	40.00	1.00	8.00
Marrone di Castel del Rio - UNIBO	Emilia-Romagna	22.22	4.50	20.00
Pelosa - UNIBO	Emilia-Romagna	50.00	3.86	38.57
Bovalghe - UNIBO	Emilia-Romagna	72.73	2.88	41.82
Pastanese - UNIBO	Emilia-Romagna	53.85	3.57	38.46
Sborga' site 2- UNIBO	Emilia-Romagna	0.00	0.00	0.00
Pastinese - MO	Emilia-Romagna	28.57	1.00	5.71
Ceppa - MO	Emilia-Romagna	30.00	4.00	24.00
Marrone di Zocca - MO	Emilia-Romagna	30.00	2.67	16.00
Carrarese - MO	Emilia-Romagna	56.25	3.89	43.75
Tosca/Garfagnana - MO	Emilia-Romagna	15.79	2.33	7.37
Madonna - MO	Emilia-Romagna	50.00	2.67	26.67
Loglia - MO	Emilia-Romagna	25.00	2.00	10.00

(Continued)

Table 2. (Continued).

Chestnut variety	Region	Chestnut brown rot		
		Incidence D (%)	Severity S	McKinney Index MI (%)
Biancherina - MO	Emilia-Romagna	46.15	2.83	26.15
Svizzero - MO	Emilia-Romagna	30.00	2.67	16.00
Pilistella - MO	Emilia-Romagna	42.86	2.67	22.86
Loiola - MO	Emilia-Romagna	44.44	2.00	17.78
Lisanese - MO	Emilia-Romagna	20.00	2.00	8.00
Salvane - MO	Emilia-Romagna	13.33	3.00	8.00
Bovalghe - MO	Emilia-Romagna	43.75	3.43	30.00
Pelosa - MO	Emilia-Romagna	77.78	3.43	53.33
Molana - MO	Emilia-Romagna	31.25	2.40	15.00
Montemarano - MO	Emilia-Romagna	66.67	3.07	40.95
Castel del Rio - MO	Emilia-Romagna	90.00	3.00	54.00
Garron Rosso - MO	Emilia-Romagna	73.68	2.21	32.63
Marron Buono - MO	Emilia-Romagna	20.00	3.25	13.00
Castagna - MO	Emilia-Romagna	5.00	1.00	1.00
Average		39.29	2.69	22.44
Castagna della Madonna	Piedmont	100.00	4.65	93.,00
Colossal	Piedmont	33.33	3.20	21.33
Marsol	Piedmont	64.71	3.82	49.41
Marlhac	Piedmont	57.14	3.25	37.14
Maridonne	Piedmont	71.43	3.40	48.57
Ishyzuki precoce	Piedmont	26.32	3.00	15.79
Ishyzuki tardiva	Piedmont	52.38	3.91	40.95
Tsukuba	Piedmont	53.85	3.57	38.46
Precoce Migoule	Piedmont	65.00	3.08	40.00
Bouche de Betizac	Piedmont	90.91	4.70	85.45
Laguepie	Piedmont	55.00	3.45	38.00
Marrubia	Piedmont	85.71	3.61	61.90
Marrone di Susa	Piedmont	75.00	3.40	51.00
Marrone di Chiusa Pesio	Piedmont	47.62	3.40	32.38
Marrone di Viterbo	Piedmont	36.84	4.57	33.68
Average		61.02	3.67	45.81

Table 3. Fungal genera isolated from a total of 360 chestnuts which were classified after visual inspection into chestnut brown rot (CBR) severity classes (0 to 5) based on the proportions of internal nut tissue deterioration (see text). Numbers in parentheses indicate the percentages.

CBR Severity class	Number of samples	Fungal genera detected (%)					
		Gnomoniopsis	*Penicillium*	*Trichoderma*	*Aspergillus*	*Alternaria*	*Fusarium*
0	60	3 (5)	0	0	0	0	0
1	60	30 (50)	2 (3)	1 (1.6)	1 (1.6)	2 (3)	2 (3)
2	60	57 (95)	3 (5)	2 (3)	1 (1.6)	2 (3)	0
3	60	59 (98.3)	2 (3)	0	0	0	4 (6.6)
4	60	42 (70)	2 (3)	1 (1.6)	3 (5)	1 (1.6)	1 (1.6)
5	60	22 (36.6)	2(3)	4 (6.6)	2 (3)	5 (8.3)	2 (3)
Total	360	213 (59.2)	11 (3)	8 (2)	7 (1.9)	10 (2.7)	9 (2.5)

Figure 4. A) (a-s). Necrotic lesions formed on chestnut cuttings (*Castanea sativa*) 20 d after inoculation with *Gnomoniopsis castaneae* isolates of haplotype A [a) 'GC_Castagna di Val di Castro-SITE 1'; b) 'GC_Barrile-BARV'; c) 'GC_Castagna bionda di Lunano'; d) 'GC_SanPietro'; e) 'GC_Castel del Rio-MO'; f) isolate 'PD-ST30'; and g) inoculation control.] Inoculations with *G. castaneae* isolates of haplotype B [h) 'GC_Marrone scuro-2710'; i) 'GC_Marrone di Chiusa Pesio'; j) 'GC_Rossera-2414'; k) 'GC_Tosca/Garfagnana'; l) 'GC_Patriarca-3595'; m) 'GC_Bouche de Betizac'; n) 'GC_Santu Giuanni-ARI3'; o) 'GC_Pilistella'; p) 'GC_Precoce Migoule'; q) 'GC_Marrone di Viterbo'; r) 'GC_Marrone di Perledo-3419'; or s) isolate 'PD-ST55'. B)] Necrosis lenghts (cm) in stems of cuttings of different chestnut varieties previously inoculated with different *Gnomoniopsis castaneae* isolates. Means accompanied by the same lowercase letter are not different ($P \geq 0.05$).

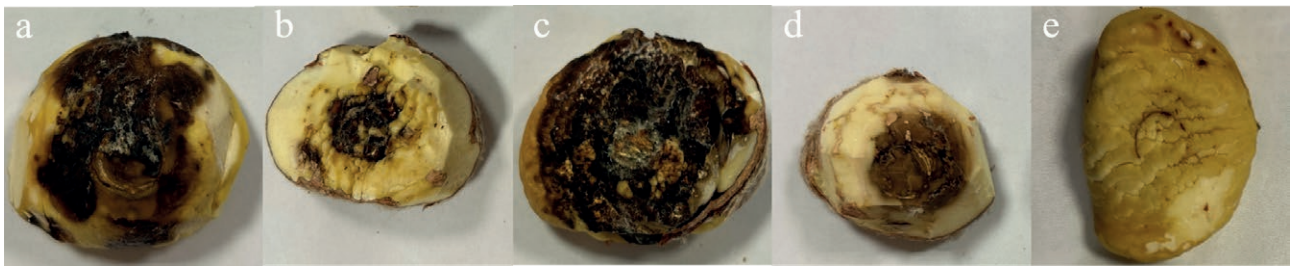

Figure 5. Necrotic lesions formed in the endosperm tissues of chestnut (*Castanea sativa*) fruits 16 d after inoculations with *Gnomoniopsis castaneae* isolates molecularly characterized as Gc-haplotype A and B based on *β-tubulin* gene sequences typing: a) 'GC_Castagna bionda di Lunano'; b) 'GC_Marrone scuro-2710'; c) 'GC_Rossera-2414'; d) 'GC_Marrone di Chiusa Pesio'; and e) the inoculation control.

castaneae isolates within each *Gc*-haplotype group (Confidence level used: 0.95, $P > 0.5$) (Figure 4B). Among the *Gc*-haplotype A isolates, the isolates 'GC_Castagna bionda di Lunano' and 'GC_Castagna di Val di Castro' site 1 (Marche) were the most aggressive, producing a longer necrosis compared to the other isolates. Within *Gc*-haplotype B, the most aggressive isolate was 'GC_Tosca Garfagnana from Emilia Romagna' (Figure 4B).

Pathogenicity test on chestnut fruits

The 16 isolates of *G. castaneae,* five of *Gc*-haplotype A and 11 of *Gc*-haplotype B, all induced necrotic lesions on fruits 16 d post inoculation (Supplementary Table 3). Mean areas of necrosis ranged from 0.84 (± 0.63) cm^2, induced by isolate 'GC_Precoce Migoule', up to 11.21 (± 5.97) cm^2 induced by isolate 'GC_Santu Giuanni_ARI3' (Figure 5). Statistical analyses showed that these data were not normally distributed, so the dataset was transformed using the function "Boxcox" and a lambda value of 0.26. With the transformed data, the ANOVA test showed no statistically significant difference ($P = 0.4787$) between necrosis caused by the two *Gc*-haplotype groups.

Pathogenicity test on chestnut seedlings under different water regimes

An additional pathogenicity test was performed inoculating the 16 *G. castaneae* isolates (*Gc*-haplotypes A and B) on 3-year-old chestnut seedlings, cultivated in two different water regimes (WR) in controlled conditions for 30 days. All 16 *G. castaneae* isolates induced cortical necrosis on seedlings (Supplementary Table 4). ANOVA tests showed no statistically significant ($P > 0.05$) differences within each water regime in necrosis produced by the different *G. castaneae* isolates (Figure 6A). Generally, necrosis induced by each isolate were longer for WR_B (150 mL $month^{-1}$) than for WR_A (150 mL $week^{-1}$). Exceptions were for 'GC_Marrone di Perledo' and 'GC_Marrone di Chiusa Pesio', where mean necrosis length for WR_A (150 mL $week^{-1}$) was 3.86 cm (± 1.91), while for the WR_B (150 mL $month^{-1}$) plants mean lesion length was 3.33 cm (± 1.83) (Supplementary Table 4).

Differences were detected in severity of necroses caused by *Gc*-haplotypes A and B. The *Gc*-haplotype A isolates produced longer necroses in plants under WR_B than for WR_A. In contrast, *Gc*-haplotype B showed no difference ($P > 0.05$) in necrosis between the two water regimes (Figure 6B).

DISCUSSION

Results from this study have demonstrated the importance of *G. castaneae* as an emerging pathogen causing diseases in European chestnut in Italy. Approximately 69% of the 1812 assessed nuts from 100 of the varieties growing in the Italian Biodiversity Repository Fields, located in Piedmont, Tuscany, Emilia-Romagna, and Campania, and in commercial orchards in Lombardy, Trentino Alto-Adige, Marche and Sardinia, were affected by fruit rots. Occasionally, pink and black rots as well as internal green mold were recorded in fruit samples. The most frequently detected postharvest alteration was brown rot of nut endosperms, although the nuts had been harvested and selected for the Fruit Exhibition showing no external symptoms, no loss of consistency, or other significant visible defects. These results indicate that visual inspection of nuts is often insufficient for an accurate assessment of brown rot and detection of *G. castaneae*, which was also reported by Vettraino *et al.* (2021). Consequently, nuts that appear externally healthy may be internally compromised and unmarketable (Shuttleworth and Guest, 2017). Reliable detection of chestnut rots currently relies on destructive methods or advanced techniques such as X-ray comput-

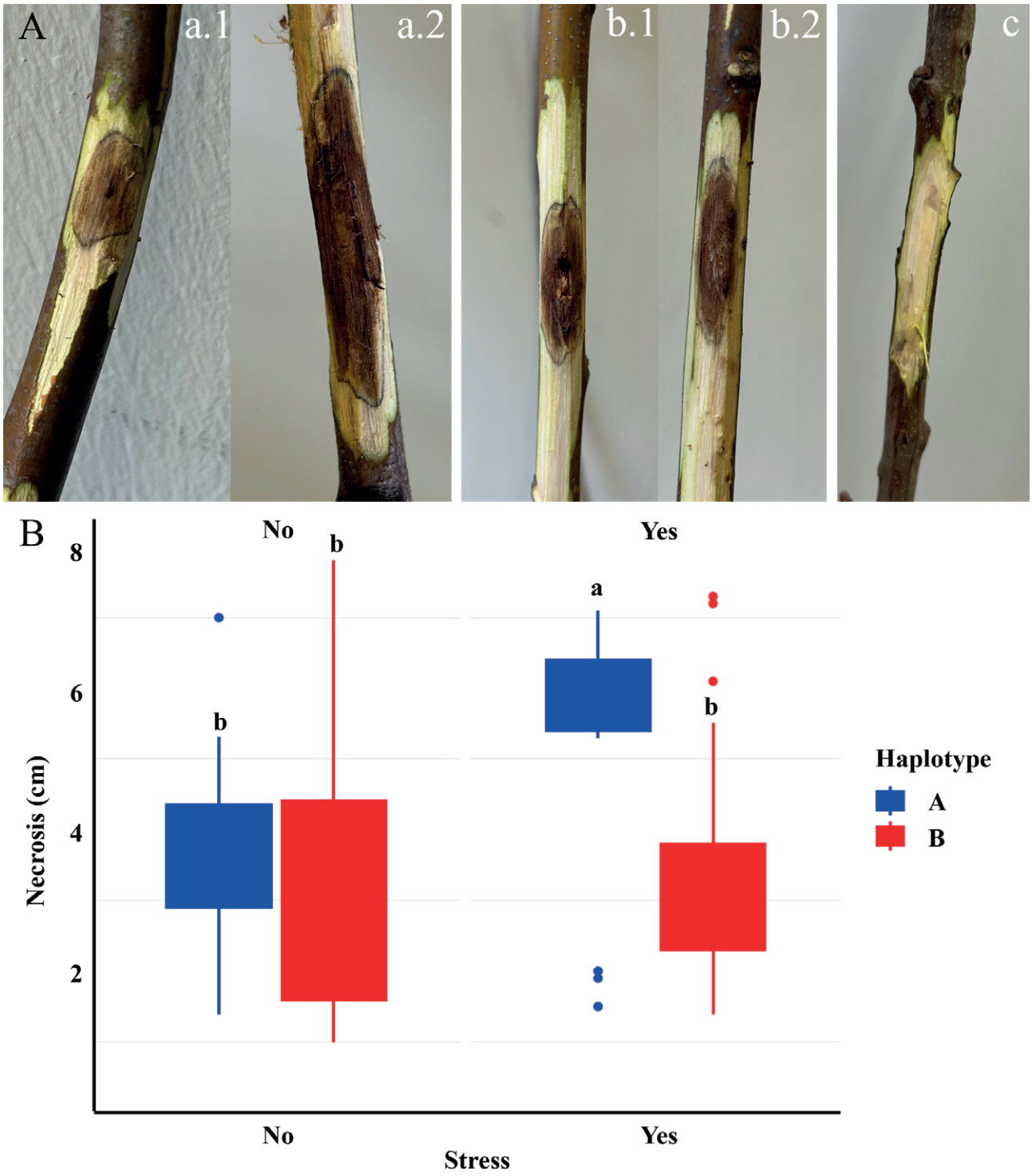

Figure 6. A) (a–c). Necrotic lesions formed on chestnut (*Castanea sativa*) seedlings 30 d after inoculations with *Gnomoniopsis castaneae* isolates. The seedlings received two different water regimes to simulate no stress or stress conditions. a.1) Haplotype A: 'GC_Castagna di Val di Castro – SITE 1', in the 150 mL week^{-1} irrigation treatment, and a-2) in the 150 mL month^{-1} treatment; b.1) 'GC_Castagna Bionda di Lunano' in the 150 mL week^{-1} irrigation treatment, and b.2) in the 150 mL month^{-1} treatment; c) experimental control. B) Lengths (cm) of necroses in stems of cuttings of different chestnut varieties that were previously inoculated with different *Gnomoniopsis castaneae* isolates. Means accompanied by the same lowercase letter are not different ($P \geq 0.05$). Statistical analysis showing the significantly different behaviour of the Haplotype A isolates when comparing necrosis under different water stress conditions.

ed tomography (CT), which provides deep penetration capability (Bernard *et al*., 2020; Matsui *et al*., 2022).

After nut bi-dissection, brown rot was detected in more than half of the nuts analyzed, with different levels of disease severity in nut endosperms. The McKinney Index gave good assessments of the impact of brown rot on chestnut fruit. This Index synthesizes incidence (D) and severity (S) of this disease. The present study results showed that average Mckinney index was 42.19%, with different Italian regional situations, ranging from 16% (Trentino Alto Adige) to 72% (Lombardy). Most of the assessed chestnut varieties had fruit brown rots. Epidemiological data from Australia, Italy and Portugal have showed that most chestnut germplasm can be affected, though severity and extent of endosperm alteration can be variable (Lione, 2016; Shuttleworth and Guest, 2017; Possamai *et al*., 2023). The present study showed that from chestnuts showing brown rot, *G. castaneae* was isolated from fruits, the endosperms of which involved from 11 to 50% of tissues (disease severity classes 2 and 3). Brown rot was not observed in a few varieties, including: 'Marrone Rugoso di Acquasanta Terme', 'Castagna di Val di Castro' site 1 and 'Marrone Classico di Acquasanta Terme' (Marche); 'Sborgà' (Emilia-Romagna); and 'Loccheddu- LOCG2' and 'Ildubba-ILDP' (Sardinia).

Gnomoniopsis castaneae was occasionally also isolated from nuts classified as McKinney Index MI = 0 (no visible episperm alterations), confirming the pathogen's latent stage, as has been previously reported by Maresi *et al*. (2013) and Dennert *et al*. (2015). This can also explain how apparently healthy nuts were found infected after 2 weeks of storage at room temperature, without any source of inoculum. This ability to latently persist, and its widespread distribution, underlies the success of this pathogen. The ubiquity and ability to infect different chestnut tree components was demonstrated by Topalidou *et al*. (2024), who detected *G. castaneae* using Bar-HRM analysis, at varying success rates from different chestnut tissues (buds, flowers, or nuts), and across multiple seasonal stages. Ability to cause damage on fruit is likely to be related to nut storage conditions, temperature shown to be important in the present study.

Factors such as pre-harvest litter management, climate conditions (Shuttleworth *et al*., 2013; Lione *et al*., 2015, 2021), and especially storage time and temperature are all likely to influence the transition from latent to active nut infections, accelerating post-harvest disease progression (Morales-Rodriguez *et al*., 2022). These factors could explain the ambiguous results obtained from the particular varieties (i.e. 'Ceppa', 'Pelosa', 'Bovalghe', 'Pastanese', 'Svizzera') cultivated in two different Germoplasm Repositories of Emilia Romagna.

Gnomoniopsis castaneae was identified in the present study using morphological characteristics and molecular assays, and ITS sequence analyses were effective for species-level resolutions. Further genotyping based on the *β-tubulin* sequences confirmed the two *G. castaneae* haplotypes (A and B) recently described by Seddaiu *et al*. (2023). The results of the present study have expanded knowledge on the geographical distribution of the two haplotypes, by obtaining information on their presence/absence in Emilia-Romagna (*Gc*-haplotypes A and B), Marche (A) and Campania (A). According to previous findings, 64% of Italian isolates belonged to *Gc*-haplotype A (mainly found in central Italy), while 36% belonged to *Gc*-haplotype B (more common in the north Italian regions) (Seddaiu *et al*., 2023). Although the two haplotypes are morphologically indistinguishable, they differ in virulence, particularly in ability to rapidly induce necroses in inoculated fruits. This was indicated by Seddaiu *et al*. (2023), after conducting pathogenic tests on chestnut fruits with seven representative Sardinian isolates of *G. castaneae* (*Gc*-haplotypes A and B). In the present study assessments conducted on Marche fruits and chestnut cuttings, inoculating 16 *G. castaneae* isolates (five of *Gc*-haplotype A and 11 of *Gc*-haplotypes B), no statistically significant differences were detected between the two *Gc*-haplotypes in their necrotic effects on fruits or chestnut cuttings. Both haplotypes induced cankers when inoculated onto cuttings, consistent results of similar damage in other *Fagaceae* hosts (Droby *et al*., 2023). Ability of *G. castaneae* to cause branch cankers and necroses was also recorded in chestnut trees in Veneto and Sardinia. From branch cankers, which turned shoot tissues from pale green to brown and withered, *G. castaneae* was commonly isolated, while *Do. iberica, C. parasitica*, *N. luteum* were only sporadically isolated. Symptoms in chestnut canopies were generally associated to *C. parasitica* (Rigling and Prospero, 2018). Cankers caused by *G. castaneae* are distinguishable from those caused by *C. parasitica*, due to their dark brown bark, while for *C. parasitica* outer bark cankers are typically red-orange with swollen and fissured host cortical tissues.

Plants, cultivated at the same temperature, but subjected to prolonged water stress and inoculated with *Gc*-haplotype A, developed more severe symptoms than those receiving regular irrigation, indicating that water stress promoted aggressiveness of *Gc*-haplotypes. From the present study data, when the pathogenicity tests were carried out on seedlings, water stress conditions did not affect the pathogenicity of *Gc*-haplotype B, but only that of *Gc*-haplotype A. On the other hand, *G. castaneae* has been frequently isolated from different tissues of healthy

plants (Ugolini *et al*., 2014; Pasche *et al*., 2016; Kolp *et al*., 2020). As suggested by Topalidou *et al*. (2024), buds and flowers can be reservoirs of the fungal inoculum, and *G. castaneae* can then shift from endophytic to pathogenic behaviour due to favourable environmental conditions for the pathogen combined with host stress induced by abiotic and/or biotic conditions. Several studies have shown that under conditions of stress, inoculation of endophytes into plant tissues can result in disease symptoms (necrosis or chlorosis) and/or growth inhibition of host plants (Schulz *et al*., 1998).

CONCLUSIONS

Gnomoniopsis castaneae has increasing impacts on chestnut hosts, as the pathogen can take advantage of conducive conditions after fruit harvest, and of abiotic stress conditions for affecting plants. The data presented here of Gc-haplotype virulence, and the recent availability of *Castanea sativa* chromosome-level genome and mitogenome (Bianco *et al.,* 2024; Villa *et al*., 2025), may provide a useful background for further studies of short and medium term adaptive disease management strategies. Detailed investigations into the mechanisms involving fungal behaviour and tree physiology in climate change context would be worthwhile.

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AUTHOR CONTRIBUTIONS

F.C. methodology, investigation, formal analysis, writing - original draft; C.B. investigation, formal analysis, writing - original draft; B.T.L. conceptualization, formal analysis investigation, formal analysis, Writing – Review & Editing funding acquisition, supervision; G.M. Writing – Review & Editing, supervision; S.M. conceptualization formal analysis, Writing – Review & Editing; funding acquisition, supervision.

LITERATURE CITED

Bassanelli C., Bischetti G.B., Chiaradia E.A., Rossi L., Vergani, C., 2013. The contribution of chestnut coppice forests on slope stability in abandoned territory: a case study. *Journal of Agricultural Engineering* 44: (s2). https://doi.org/10.4081/jae.2013.254

Battisti A., Bevegnu I., Colombari F., Haack R.A., 2014. Invasion by the chestnut gall wasp in Italy causes significant yield loss in *Castanea sativa* nut production. *Agricultural and Forest Entomology* 16: 75–79. https://doi.org/10.1111/afe.12036

Bianco L., Fontana P., Marchesini A., Torre S., Moser M., … Palmieri L., 2024. The *de novo*, chromosome-level genome assembly of the sweet chestnut (*Castanea sativa* Mill.) Cv. Marrone Di Chiusa Pesio. *BMC Genomic Data* 25(1): 64. https://doi.org/10.1186/s12863-024-01245-7

Bernard A., Hamdy S., Le Corre L., Dirlewanger E., Lheureux, F., 2020. 3D characterization of walnut morphological traits using X-ray computed tomography. *Plant Methods* 16(1): 115. https://doi.org/10.1186/s13007-020-00657-7

Conedera M., Krebs P., Tinner W., Pradella M., … Torriani D., 2004. The cultivation of *Castanea sativa* (Mill.) in Europe, from its origin to its diffusion on a continental scale. *Vegetation History and Archaeobotany* 13(3): 161–179. https://doi.org/10.1007/s00334-004-0038-7

Dar M.A., Rai M., 2015. *Gnomoniopsis smithogilvyi*, a canker causing pathogen on *Castanea sativa*: First report. *Mycosphere* 6(3): 327–336. https://doi.org/10.5943/mycosphere/6/3/8

Dennert F.G., Broggini G.A.L., Gessler C., Storari M., 2015. *Gnomoniopsis castaneae* is the main agent of chestnut nut rot in Switzerland. *Phytopathologia Mediterranea* 54: 199–211. https://doi.org/10.14601/Phytopathol_Mediterr-14712

Dobry E., Campbell M., 2023. *Gnomoniopsis castaneae*: an emerging plant pathogen and global threat to chestnut systems. *Plant Pathology* 72: 218–231. https://doi.org/10.1111/ppa.13670

Doyle J.J., Doyle J.L., 1990. Isolation of plant DNA from fresh tissue. *Focus* 12: 13–15.

Donis González I.R., Fulbright D.W., Ryser E.T., Guyer D., 2009. Shell mold and kernel decay of fresh chestnuts in Michigan. In *I European Congress on Chestnut-Castanea 2009* 866: 353-362. https://doi.org/10.17660/ActaHortic.2010.866.45

European and Mediterranean Plant Protection Organization, 2017. EPPO Reporting Service No. 02–2017 Num. Article: 2017/047. Available online: http://archives.eppo.int/EPPOReporting/2017/Rse-1702.pdf (accessed 22 March 2021).

Freitas T.R., Santos J.A., Silva A.P., Fraga H., 2021. Influence of climate change on chestnut trees: a review. *Plants* 10: 1463. https://doi.org/10.3390/plants10071463

Gabrielli A., 1994. La civiltà del castagno. *Monti e boschi* 65: 3.

Gaffuri F., Longa C.M.O., Turchetti T., Danti R., Maresi G., 2017. 'Pink rot': infection of *Castanea sativa* fruits by *Colletotrichum acutatum*. *Forest Pathology* 47: e12307. https://doi.org/10.1111/efp.12307

Glass N.L., Donaldson G.C., 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61: 1323–1330. https://doi.org/10.1128/AEM.61.4.1323-1330.1995

González I.R., Guyer D.E., Fulbright D.W., 2016. Quantification and identification of microorganisms found on shell and kernel of fresh edible chestnuts in Michigan. *Journal of the Science of Food and Agriculture* 96: 4514–4522. https://doi.org/10.1002/jsfa.7667

Kolp M., Double M. L., Fulbright D. W., MacDonald W. L., Jarosz A. M., 2020. Spatial and temporal dynamics of the fungal community of chestnut blight cankers on American chestnut (*Castanea dentata*) in Michigan and Wisconsin. *Fungal Ecology* 45:100925. https://doi.org/10.1016/j.funeco.2020.100925

Lema F., Baptista P., Oliveira C., Ramalhosa E., 2023. Brown rot caused by *Gnomoniopsis smithogilvyi* (syn. *Gnomoniopsis castaneae*) at the level of the chestnut tree (*Castanea sativa* Mill.). *Applied Sciences* 13: 3969. https://doi.org/10.3390/app13063969

Linaldeddu B.T., Deidda A., Scanu B., Franceschini A., Alves A., ... Phillips A.J.L., 2016. Phylogeny, morphology and pathogenicity of *Botryosphaeriaceae, Diatrypaceae* and *Gnomoniaceae* associated with branch diseases of hazelnut in Sardinia (Italy). *European Journal of Plant Pathology* 146(2): 259–279. https://doi.org/10.1007/s10658-016-0912-z

Lione G., Giordano L., Sillo, F., Gonthier P., 2015. Testing and modelling the effects of climate on the incidence of the emergent nut rot agent of chestnut *Gnomoniopsis castanea*. *Plant Pathology* 64: 852–863. https://doi.org/10.1111/ppa.12319

Lione G.G., 2016. Ecology and epidemiology of the emerging plant pathogen *Gnomoniopsis castaneae*. *Journal of Plant Pathology* 98(4): S5-S5.

Lione G., Danti R., Fernandez-Conradi P., Ferreira-Cardoso J.V., Lefort F., ... Gonthier P., 2019. The emerging pathogen of chestnut *Gnomoniopsis castaneae*: the challenge posed by a versatile fungus. *European Journal of Plant Pathology* 153: 671–685. https://doi.org/10.1007/s10658-018-1597-2

Lione G., Giordano L., Sillo F., Brescia F., Gonthier P., 2021. Temporal and spatial propagule deposition patterns of the emerging fungal pathogen of chestnut *Gnomoniopsis castaneae* in orchards of north-western Italy. *Plant Pathology* 70(9): 2016–2033. https://doi.org/10.1111/ppa.13451

Marais A., Murolo S., Faure C., Brans Y., Larue C., ... Candresse, T., 2021. Sixty years from the first disease description, a novel badnavirus associated with chestnut mosaic disease. *Phytopathology* 111(6): 1051–1058. https://doi.org/10.1094/PHYTO-09-20-0420-R

Maresi G., Oliveira Longa C.M., Turchetti T., 2013. Brown rot on nuts of *Castanea sativa* Mill: an emerging disease and its causal agent. *iForest* 6: 294–301. https://doi.org/10.3832/ifor0952-006

Mattioli W., Mancini L. D., Portoghesi L., Corona P., 2016. Biodiversity conservation and forest management: The case of the sweet chestnut coppice stands in Central Italy. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology* 150(3): 592-600. https://doi.org/10.1080/11263504.2015.1054448

Matsui H., 2022. Biological Sensing Using Infrared. *Biosignal Processing* 209.

McKinney H.H., 1923. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. *Journal of Agricultural Research* 26: 195–218.

Morales-Rodriguez C., Bastianelli G., Caccia R., Bedini G., Massantini R., ... Vannini A., 2022. Impact of 'brown rot' caused by *Gnomoniopsis castaneae* on chestnut fruits during the post-harvest process: critical phases and proposed solutions. *Journal of the Science of Food and Agriculture* 102: 680–687. https://doi.org/10.1002/jsfa.11397

Nicoletti R., Beccaro G.L., Sekara A., Cirillo C., Di Vaio C., 2021. Endophytic fungi and ecological fitness of chestnuts. *Plants* 10: 542. https://doi.org/10.3390/plants10030542

Pasche S., Calmin G., Auderset G., Crovadore J., Pelleteret P., ... Lefort F., 2016. *Gnomoniopsis smithogilvyi*

causes chestnut canker symptoms in *Castanea sativa* shoots in Switzerland. *Fungal Genetics and Biology* 87: 9–21. https://doi.org/10.1016/j.fgb.2016.01.002

Pérez-Girón J.C., Álvarez-Álvarez P., Díaz-Varela E.R., Mendes Lopes D.M., 2020. Influence of climate variations on primary production indicators and on the resilience of forest ecosystems in a future scenario of climate change: application to sweet chestnut agroforestry systems in the Iberian Peninsula. *Ecological Indicators* 113: 106199. https://doi.org/10.1016/j.ecolind.2020.106199

Pezzi G., Ferretti F., Maltoni A., Krebs P., Conedera M., Maresi G., 2022. The chestnut orchards in the Bolognese Apennines: A vanishing socio-ecological habitat. In *Historical Ecology: Learning from the Past to Understand the Present and Forecast the Future of Ecosystems* pp. 195–205.

Possamai G., Dallemole-Giaretta R., Gomes-Laranjo J., Sampaio A., Rodrigues P., 2023. Chestnut brown rot and *Gnomoniopsis smithogilvyi*: Characterization of the causal agent in Portugal. *Journal of Fungi* 9(4): 401. https://doi.org/10.3390/jof9040401

Prada M., Bravo F., Berdasco L., Canga E., Martínez-Alonso C., 2016. Carbon sequestration for different management alternatives in sweet chestnut coppice in northern Spain. *Journal of Cleaner Production* 135: 1161-1169. https://doi.org/10.1016/j.jclepro.2016.07.041

Prospero S., Heinz M., Augustiny E., Chen Y. Y., Engelbrecht J., … Fonti P., 2023. Distribution, causal agents, and infection dynamic of emerging ink disease of sweet chestnut in Southern Switzerland. *Environmental Microbiology* 25(11): 2250–2265. https://doi.org/10.1111/1462-2920.16455

Rigling D., Prospero S., 2018. *Cryphonectria parasitica*, the causal agent of chestnut blight: invasion history, population biology and disease control. *Molecular Plant Patholo*gy 19(1): 7–20. https://doi.org/10.1111/mpp.12542

Rodrigues P., Driss O.J., Gomes-Laranjo J., Sampaio A., 2022. Impact of cultivar, processing and storage on the mycobiota of European chestnut fruits. *Agriculture* 12: 1930. https://doi.org/10.3390/agriculture12111930

Santos M.J., Pinto T., Vilela A., 2022. Sweet chestnut (*Castanea sativa* Mill.) nutritional and phenolic composition interactions with chestnut flavor physiology. *Foods* 11: 4052. https://doi.org/10.3390/foods11244052

Schulz B., Guske S., Dammann U., Boyle C., 1998. Endophyte host interactions II. Defining symbiosis of the endophyte-host interaction. *Symbiosis* 25: 213–227.

Seddaiu S., Mello A., Sechi C., Cerboneschi A., Linaldeddu B.T., 2021. First Report of *Neofusicoccum parvum* associated with chestnut nut rot in Italy. *Plant Disease* 105: 3743. https://doi.org/10.1094/PDIS-01-21-0072-PDN

Seddaiu S., Mello A., Sarais L., Mulas A., Sechi C., Ruiu P.A., … Linaldeddu B.T., 2023. Haplotypes distribution and virulence of *Gnomoniopsis castaneae* in Italy. *Journal of Plant Pathology* 105: 1135–1140. https://doi.org/10.1007/s42161-023-01459-1

Shuttleworth L.A., 2012 . Fungal planet description sheet 108: *Gnomoniopsis smithogilvyi* LA Shuttleworth, ECY Liew & DI Guest, sp. nov. *Persoonia* 28: 142–143. https://doi.org/10.3767/003158512X652633

Shuttleworth L.A., Liew E.C.Y., Guest D.I., 2013. Survey of the incidence of chestnut rot in south-eastern Australia. *Australasian Plant Pathology* 42: 63–72. https://doi.org/10.1007/s13313-012-0170-2

Shuttleworth L.A., Guest D.I., 2017. The infection process of chestnut rot, an important disease caused by *Gnomoniopsis smithogilvyi* (*Gnomoniaceae, Diaporthales*) in Oceania and Europe. *Australasian Plant Pathology* 46(5): 397–405. https://doi.org/10.1007/s13313-017-0502-3

Suna S., Avşar B., Koçer S., Çopur Ö.U., 2021. Effects of different pretreatments on the physicochemical characteristics and quality criteria of chestnut (*Castanea sativa* Mill.) pickle: a new value-added product. *Journal of Food Processing and Preservation* 45:e15669. https://doi.org/10.1111/jfpp.15669

Topalidou E., Lagiotis G., Bosmali I., Stefanidou E., Tsirogiannis D., Vettraino A.M., Madesis P., 2024. Water stress enhances the pathogenicity of *Gnomoniopsis castaneae* in chestnut trees. *Journal of Plant Pathology* 106: 345–352. https://doi.org/10.1016/j.funbio.2024.06.003

Ugolini F., Massetti L., Pedrazzoli F., Tognetti R., Vecchione A., … Maresi G., 2014. Ecophysiological responses and vulnerability to other pathologies in European chestnut coppices, heavily infested by the Asian chestnut gall wasp. *Forest Ecology and Management* 314: 38–49. https://doi: 10.1016/j.foreco.2013.11.031

Vettraino A. M., Luchi N., Rizzo D., Pepori A. L., Pecori F., Santini A., 2021. Rapid diagnostics for *Gnomoniopsis smithogilvyi* (syn. *Gnomoniopsis castaneae*) in chestnut nuts: new challenges by using LAMP and real-time PCR methods. *AMB Express* 11(1): 105. https://doi.org/10.1186/s13568-021-01266-w

Villa S., Marchesini A., Torre S., Bianco L., Fontana P., De Quattro C., … Sebastiani F., 2025. First complete mitogenome assembly of *Castanea sativa*: structure,

comparative genomics, and phylogeny. *Tree Genetics & Genomes* 21(4): 22. https://doi.org/10.1007/s11295-025-01707-8

Visentin I., Gentile S., Valentino D., Gonthier P., Tamietti G., Cardinale F., 2012. *Gnomoniopsis castaneae* sp. nov. (*Gnomoniaceae*, *Diaporthales*) as the causal agent of nut rot in sweet chestnut. *Journal of Plant Pathology* 94: 411–419. http://www.jstor.org/stable/45156050.

Washington W.S., Allen A.D., Dooley L.B., 1997. Preliminary studies on *Phomopsis castanea* and other organisms associated with healthy and rotted chestnut fruit in storage. *Australasian Plant Pathology* 26: 37–43. https://doi.org/10.1071/AP97006

White T.J., Bruns T., Lee S., Taylor J.W., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: a guide to methods and applications*. (Innis M.A., Gelfand D.H., Sninsky J.J., White T.J. ed.). San Diego, C, USA, Academic Press, 315–322.