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Review

Brown Rot Caused by *Gnomoniopsis smithogilvyi* (syn. *Gnomoniopsis castaneae*) at the Level of the Chestnut Tree (*Castanea sativa* Mill.)

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Featured Application: The findings of the present review study can be of great importance for European chestnut producers. The causal agent of brown rot causes significant economic losses and increases the amount of waste, not meeting sustainability principles.

Abstract: The European chestnut tree (*Castanea sativa* Mill.) has great economic importance, mostly due to the recognized nutritional value of its fruit. Thus, the development and improvement of the techniques of the production, preservation, and control of the diseases/pests of chestnut trees is a topic of great interest to producers, companies, researchers, and consumers to ensure the quality of this exceptional fruit. Recently, an emerging rot in chestnuts caused by the fungus *Gnomoniopsis smithogilvyi* (syn. *Gnomoniopsis castaneae* Tamietti) (Gnomoniaceae, Diaporthales) was reported both in Australia and Europe. Since then, the number of records of this pathogen in several countries of the world (Europe and Asia) where *Castanea* spp. is cultivated has been increasing. This disease, called "brown rot", has been causing significant production losses, raising serious concerns for producers and the chestnut industry. This review describes the world distribution and life cycle of the causal agent of brown rot. The life cycle of *G. smithogilvyi* can involve primary infection, caused by ascospores, and secondary infection, related to the asexual phase of the fungus (conidia). Then, the analytical methods used to detect *G. smithogilvyi* are described. Furthermore, the incidences of the disease caused by *G. smithogilvyi* are presented, ranging from 5 to 94%, with high infection rates causing significant economic losses. The damages caused by *G. smithogilvyi* are discussed. In fact, it can act as an endophyte or as a pathogenic fungus, causing fruit rot, canker in several plant tissues, and necrosis in leaves, as well as in galls caused by the gall wasp *Dryocosmus kuriphilus* Yasumatsu. Possible pre- and post-harvest methods to mitigate the damage caused by moulds, and in particular *G. smithogilvyi*, are presented, including biocontrol agents and chemicals. Finally, some challenges and future prospects for a number of uncertainties related to the epidemiology, geographic distribution, spread, detection, and management of this disease are discussed.

Keywords: *Gnomoniopsis castaneae*; emerging disease; fruit rot; damages; diagnostic methods

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1. Introduction

The chestnut is a fruit of great interest in many regions of the world, with a total production of approximately 2.3 million tonnes, with China being the largest producer, with 1,743,354 tonnes [1]. However, this country produces the species *Castanea mollissima* Blume, while European countries produce *Castanea sativa* Mill. In the worldwide and European chestnut productions, Portugal ranks in the seventh and fourth positions, respectively, with an annual production of 42,180 tonnes in 2020, distributed through 51,700 ha [1].

The production of chestnuts in Portugal has a significant economic impact, especially in the region of Trás-os-Montes, where the largest area of cultivation of the chestnut tree is concentrated (30,000 ha) [2]. However, in the last decades, the production of chestnuts in this region, as well as in other regions of the world, has greatly declined. Specifically, in the Trás-os-Montes region (Portugal), losses of 70% in the production of some chestnut varieties have been reported in some areas for the year 2022 [3]. Both abiotic and, particularly, biotic stress factors, such as pests (ex. *Curculio* spp. and *Cydia* spp.) and diseases (ex. canker), have been the main causes of this decline [4]. *Curculio* spp. and, in particular, *Curculio elephas* (an important pest of the European chestnut *Castanea sativa* [5]) may cause significant economic damages, approximately 25–30% per year in Turkey, as mentioned by Yaman et al. [6]. Furthermore, on average, 56.3 and 27.8% of the infested fruits contain one or two immatures (eggs and larvae) [7], showing the frequent presence of this chestnut weevil in the groves. More recently, when analyzing the loss of fruit traits due to damage by this weevil in four populations of healthy sweet chestnut trees in Turkey, Caliskan et al. [8] verified that the fruit weight loss percentages varied between 25% and 32%, demonstrating again the production and economic losses that this pest can cause to producers. *Cydia splendana* Hübner (Lepidoptera: Tortricidae) is another pest that chestnut producers face because the larvae feed on the fruits, reducing their quality and economic value. Significant attacks in Spain [9] and Hungary have been reported. Nevertheless, management strategies to control chestnut tortrix in Italy [10] and Hungary [11,12] have been developed.

Concerning diseases, the chestnut blight fungus *Cryphonectria parasitica* causes cankers, lesions caused by the growth of mycelia within the bark tissue of the host plant, which in some extreme situations may result in the death of all plant tissue distal to the canker [13]. This disease is frequently controlled using hypovirulent strains [14]. However, some challenges must be surpassed because it is known that the presence of other fungi in cankers may inhibit the hypovirulent form of the pathogen more than the virulent form, intensifying the canker development [13]. Moreover, ink disease is caused by the oomycete *Phytophthora cinnamomi*, which is a dangerous pathogen that causes root rot, threatening the production of chestnuts worldwide [15,16].

In the Trás-os-Montes region, this situation becomes even worse due to the newly introduced pest *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) and, more recently, the opportunistic fungal pathogen *Gnomoniopsis smithogilvyi* (syn. *Gnomoniopsis castaneae*) [4,17]. Dobry and Campbell [18] reported that the origin of the disease is not known. In Portugal, the presence of this fungus in chestnuts was reported for the first time in 2020 by Possamai [19] and then by Coelho and Gouveia [20]. Despite being an emerging disease in chestnuts, the presence of this fungus has already been reported in several European countries, as well as in Asia and Australia, being mainly responsible for the brown rot of chestnut tissues. The disease caused by brown rot in chestnut tissues is still difficult to understand. The fungus presence is not visible outside the fruit, even after it has already rotted inside. Nevertheless, *G. smithogilvyi* can be present in chestnut tissues without causing symptoms. However, due to the high incidence of the disease and because it is difficult to control and manage, *G. smithogilvyi* is now recognized as an emerging pathogen, threatening the production of chestnuts and challenging researchers, policymakers, and chestnut producers on a global scale. Dobry and Campbell [18] referred to this fungus as a ubiquitous endophyte; however, it may be shifted to pathogenic activity due to climate change. Under this premise, the purpose of this review is to provide a comprehensive view of the state of the art of the causal agent of this disease, highlighting the incidences of the disease and damages caused by this fungus, as well as to present potential strategies that can be applied for its management.

2. *Gnomoniopsis smithogilvyi*—Biology, Epidemiology, Symptomatology, and Identification

G. smithogilvyi is an ascomycete with a wide distribution, occurring in several countries in Europe, Asia, Australia, and America [21–23]. It has been reported to exhibit diverse lifestyles as an endophyte, inhabiting different asymptomatic chestnut tree plant tissues,

and as a pathogen, causing "brown rot" in fruits [22,24,25]. *G. smithogilvyi* can also cause cankers, as reported by Dar and Rai [26] in India, Lewis et al. [27] in the United Kingdom, Trapiello et al. [28] in Spain, O´Loinsigh et al. [29] in Ireland, and Aglietti et al. [30]. In fact, the bark canker caused by *G. smithogilvyi* shows a symptomatology very similar to that caused by the chestnut blight pathogen *Cryphonectria parasitica*, which is the causal agent of chestnut canker [31], but the severity may be distinct. *G. smithogilvyi* has also been reported as a pathogen of several other species, affecting mainly nuts (hazelnuts (*Corylus avellana* L.), manna ash (*Fraxinus ornus* L.), holm oak (*Quercus ilex* L.), Turkey oak (*Quercus cerris* L.), and maritime pine (*Pinus pinaster* Aiton)), causing fruit rot, cankers, and necrosis in branches and leaves [18,21,22,24,25,31,32].

This fungus was initially identified independently by Shuttleworth et al. [33] and Visentin et al. [21] as *G. smithogilvyi* and *Gnomoniopsis castaneae* (originally called *G. castanea*), respectively. However, morphological and phylogenetic analyses (based on DNA sequencing) showed the synonymy between the two taxons [34]. Currently, *G. smithogilvyi* is the name adopted for this fungus [35]. Nevertheless, as stated by Sillo et al. [36], more data on the phylogeography of this fungus are needed. In fact, its origin and taxonomical features still remain unclear, causing debate about the possible existence of different lineages and the legitimate name of the species.

The life cycle of *G. smithogilvyi* and the process of how to infect chestnut have not yet been fully known. According to Shuttleworth and Guest [37], the primary infection is caused by ascospores that spend the winter on the infected plant debris lying on the soil surface of chestnut groves. During the flowering period, these ascospores are transported to flowers, leaves, and branches by wind and wind-driven rain and insects [22] (Figure 1). The fungus infects only the female part of the flower (pistil) through the stigma–style and not through the ovary wall [37]. The appearance of multiple embryos in the fruit, where one is rotted and the other healthy, suggests that the pellicle surrounding each embryo is impervious to the fungus [37]. Additionally, the infection of female flowers may also occur via *G. smithogilvyi*-infected pollen. The main means of entry into the other plant organs, such as branches and leaves, is through wounds caused by mechanical or natural injuries [26].

Ascospore chestnut blossom infection is affected by several abiotic and biotic factors. The abiotic factors include temperature, rain, relative humidity, and wind. For example, an increase in temperature was reported to lead to a higher incidence of brown rot [38]. Likewise, Lione et al. [39] found that the propagule deposition rate of the *G. smithogilvyi* varies across seasons, being positively correlated with temperature, growing degree days at 0 and 5 °C thresholds, and wind gusts. Thus, it is expected that rising temperatures and strong winds due to climate change may increase the spread of the fungus in the future. This can be a problem because Arunrat et al. [40] predict that precipitation and the maximum and minimum temperatures will increase during three future periods, namely, near (2015–2039), mid (2040–2069), and far future (2070–2100), which may increase the incidence of the disease. These authors even suggest which major crops should be grown in order to reduce the negative impact of future climate changes, suggesting that the adaptation strategies of the cropping systems must be considered. Furthermore, Gullino et al. [41] reported that higher temperatures will allow the introduction and settlement of pests. Moreover, market globalization and transport, also linked with rising temperatures, had created favourable conditions for those pests. Therefore, in managing these pests, sustainability must always be considered.

Secondary infection, related to the asexual phase of the fungus (conidia), is not yet sufficiently studied. However, it is thought to be related to the attacks of the wasp *D. kuriphilus*, known as the chestnut gall wasp, and responsible for forming galls in the branches and leaves of chestnut trees [42,43]. Indeed, there are several reports of the presence of *G. smithogilvyi* conidia in these galls, which can be an inoculum source and thus promote fungus dispersion [42,43]. Although the role of this wasp on the *G. smithogilvyi* epidemiology is not yet documented, it was suggested that the stress induced on chestnut by *D. kuriphilus*

attacks could lead to the transition of the endophyte *G. smithogilvyi*, naturally present in chestnut tissues, from a latent mode to an active virulent pathogen [44]. Likewise, Turco et al. [45] reported that the endophytic lifestyle of *G. smithogilvyi* may play a fundamental role in its epidemiology, allowing the asymptomatic colonization of the host tissues by the fungus.

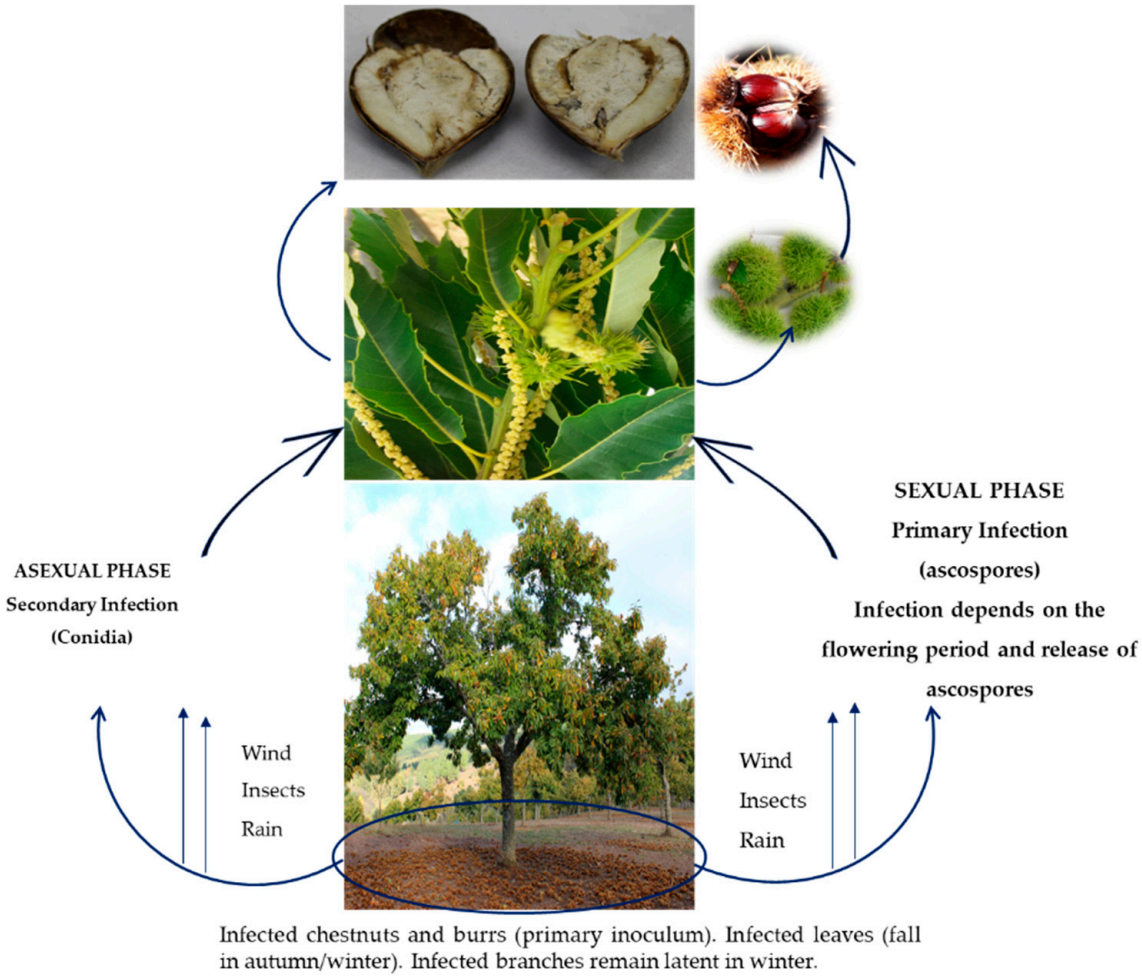

Figure 1. Process of infection of the fungus *Gnomoniopsis smithogilvyi*. Source: Adapted from Shuttleworth and Guest [37].

The detection and accurate identification of *G. smithogilvyi* are critical steps for its understanding and control. Table 1 shows the methods most used in the detection of *G. smithogilvyi*. Most of these methods require the isolation of the fungus from previously surface-disinfected asymptomatic or symptomatic fruits in culture media, such as MEA (malt extract agar), MYA (malt yeast agar), or PDA (potato dextrose agar) media [22]. Furthermore, the fungus isolation can also be carried out from *D. kuriphilus* galls, *G. smithogilvyi* fruiting bodies on galls, chestnut branch tissues, chestnut gems and flowers, and chestnut burrs. The fungal isolates obtained are then identified mainly through molecular techniques. The identification based on the morphological features of the fungus may not be conclusive due to the similarities in the vegetative and reproductive structures between *G. smithogilvyi* and other closely related species colonizing the same plant tissues. Nevertheless, some authors [34] reported the morphological-based identification by analyzing and measuring conidia, fruitbodies, and mycelium.

Table 1. Methods of diagnosis of *G. smithogilvyi* in European chestnut (*Castanea sativa* Mill.).

Diagnostic Method	Target Gene/Morphological Features	Method Sensitivity	Chestnut Tissues Where the Method Was Applied	Reference
Morphological-based				
Colonies in Potato Dextrose Agar medium	Creamy white or light brown with diffused to regular margins, and slimy conidial creamy mass drops on the surface. Woolly to felty mycelium with development in concentric circles.		Symptomatic or asymptomatic burrs and nuts	[23,46]
			Cankered bark	[26]
Conidiomata	Brownish to black, 200 × 158 µm		In fungal cultures	[42]
Conidia	Hyaline, oval, obovoid, fusoid and multigutulate, and 5.07 to 9.01 × 1.9 to 4.38 µm in size.		In fungal cultures	[23,46]
Molecular-based				
PCR	Internal transcribed spacers (ITS) region (ITS1-5.8S-ITS2)		Mycelium *	[20,23,26,42,46–48]
PCR	ITS region (168 bp) with specific primers developed for G. smithogilvyi		Mycelium *	[38]
PCR	Large subunit (LSU)		Mycelium *	[48]
PCR	Translation elongation factor 1-alpha (TEF1-α)		Mycelium *	[23,42,46–48]
PCR	Beta-tubulin (β-tubulin)		Mycelium *	[46–48]
PCR	Calmodulin		Mycelium *	[47]
PCR	RNA polymerase II (RPB2-1 and RPB2-2)		Mycelium *	[48]
Multiplex PCR	ITS, TEF1-α, β-tubulin	5–50 fg/µL	Chestnut fruit	[49]
Real-time PCR	TEF1-α	40 fg of pure fungal DNA	Chestnut fruit, leaves and twigs	[45]
	TEF1-α	0.128 pg/µL	Chestnut fruit	[50]
High-Throughput Sequencing	ITS1		Chestnut fruit	[45]
Visual-Loop-mediated isothermal amplification (V-LAMP)	TEF1-α	0.64 pg/µL	Chestnut fruit	[50]
Portable real-time LAMP (P-LAMP)	TEF1-α	0.128 pg/µL	Chestnut fruit	[50]

Data for the years 2008 and 2009. Data for the years 2017, 2018, and 2019. * Amplified region with specific primers developed for *G. smithogilvyi*.

The molecular identification of *G. smithogilvyi* is mostly based on the sequencing of a segment of the encoding ribosomal RNA genes, which includes the entire internal transcribed spacer region (ITS) (ITS1-5.8S-ITS2), or the 28S gene of the large ribosomal subunit (LSU) (Table 1). The sequencing of various protein-coding genes has been used together with the ITS region. Among protein-coding markers, the second largest (RPB2) subunits of RNA polymerase, translation elongation factor 1-alpha (EF1-α), calmodulin, and beta-tubulin have been used to identify *G. smithogilvyi* reliably (Table 1). The technique of real-time PCR (qPCR) also proved to be effective in detecting and quantifying *G. smithogilvyi*

in symptomatic and/or asymptomatic fruits, leaves, and branches of the chestnut tree [45,50]. More recently, the Loop-mediated isothermal AMPlification (LAMP) technique has been successfully used for the detection of *G. smithogilvyi* on chestnuts. Compared with the PCR and qPCR methods, the advantages of LAMP are: (i) it can be applied in the field [18]; (ii) it is faster and more user-friendly [18]; (iii) it requires less reagents; and (iv) it is more resistant to inhibitions, allowing for crude extract processing. As for the qPCR method, LAMP does not require prior isolation of the pathogen in a culture medium to detect it in the infected tissues [18,50]. Recently, a multiplex PCR was developed by Silva-Campos et al. [49] to detected the presence of *G. smithogilvyi* in chestnut fruit. This new method, based on the amplification of ITS, EF1-α, and beta-tubulin, is used as an internal control of the *C. sativa* gene *petD* in order to confirm that the negative results are due to the absence of *G. smithogilvyi* gDNA and not due to a reaction failure.

This fungus was identified by our research group this year (2022) in burrs and fruit shells collected in the Trás-os-Montes region (northeast of Portugal) (Figure 2). The identification of the fungus was made by sequencing the entire ITS region (ITS1-5.8S-ITS2), using the universal primers ITS1 and ITS4 [51].

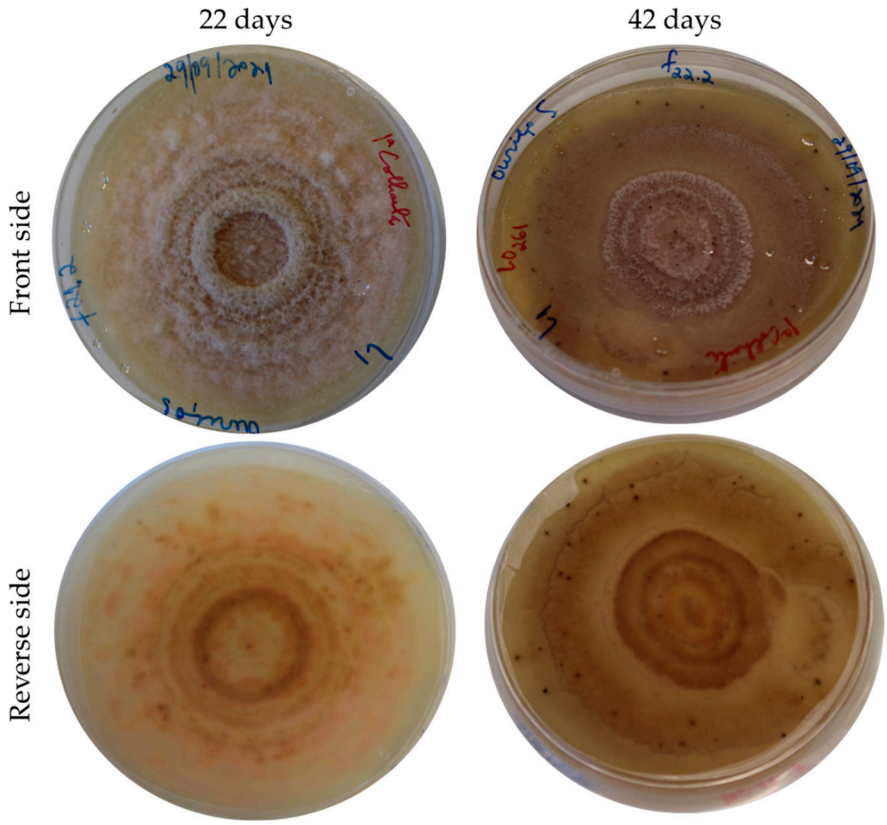

Figure 2. *Gnomoniopsis smithogilvyi* isolated from the fruit shell after 22 and 42 days of cultivation in a PDA medium.

Table 2 compiles the main symptoms associated with this fungus when exhibiting pathogenic features, but also highlights the common presence of this fungus within several asymptomatic host tissues as an endophyte. This fact can lead to an underestimation of the infection values when the diagnosis is made solely by a visual inspection of the plant tissues/fruits, not including isolation or molecular detection methods.

Table 2. Host of *G. smithogilvyi* in several species of *Castanea*, observed symptoms/asymptomatic, and incidence of the disease (%) (Source: Adapted from Lione et al. [22] and updated).

Continent	Country	Host	Observed Symptoms/Asymptomatic	Incidence of the Disease (%)	Reference
AMERICA	Chile	*C. sativa*	Asymptomatic fruits	3.7–6.5	[8]
	USA	*C. mollissima* *C. sativa* × *C. crenata*	Fruit rot		[52]
ASIA	Australia & New Zealand	*C. sativa* *C. sativa* × *C. crenata*	Fruit rot		[53]
	Australia	*Castanea* spp.	Fruit rot		[54]
		Castanea spp.	Fruit rot; asymptomatic in dead burrs		[55]
		C. sativa *C. crenata* × *C. sativa*	Fruit rot; asymptomatic in the other parts of the tree, such as female flowers, male flowers, pedicels, petioles of terminal leaves, and bark		[37]
		C. sativa	Fruits	46	[49]
	India	*C. sativa*	Canker in shoots, stems, and branches	33–58	[26]
	Turkey [1]	*C. sativa*	Fruit	19	[56]
EUROPE	Belgium	*C. sativa*	Bark canker		[57]
	Croatia	*C. sativa* × *C. crenata*	Fruits	75	[58]
	France, Italy & Switzerland	*C. sativa*	Fruit rot; asymptomatic in bark and floral shoots (artificial inoculation)	80	[21]
	France, Italy & Switzerland	*C. sativa*	Fruit rot		[36]
	France and Italy	*C. sativa*	Fruit rot		[38]
	Ireland	*C. sativa*	Canker in branches		[29]
	Italy	*C. sativa*	Fruit rot; asymptomatic in other parts of the plant (e.g., pistils and flowers, developing fruits, and external tissues of the burr)		[59]
		Castanea spp.	Necrosis in leaves and galls of *D. kuriphilus*, blight symptoms in the branches (artificial inoculation)		[60]
		C. sativa	Fruit rot; asymptomatic in bark and young shoots	8–49/2–24 [2]	[42]
		Castanea spp.	Not specified in the galls of *D. kuriphilus*		[61]
		C. sativa	Asymptomatic in shoots and galls of *D. kuriphilus*		[44]
		C. sativa	Fruit rot		[62]
		C. sativa	Fruit rot; asymptomatic in ripe fruits; non-specific in the galls of *D. kuriphilus*		[63]
		C. sativa	Necrosis in galls of *D. kuriphilus*; asymptomatic in leaves		[24,25]
		C. sativa	Necrosis or asymptomatic in galls of *D. kuriphilus*	68	[64]

Table 2. *Cont.*

Continent	Country	Host	Observed Symptoms/Asymptomatic	Incidence of the Disease (%)	Reference
		C. sativa	Fruit rot; necrosis in the galls of *D. kuriphilus;* asymptomatic in bark, shoots, leaves, galls of *D. kuriphilus*, and fruits	80	[43,65]
		C. sativa	Fruit rot; asymptomatic fruits		[50]
		C. sativa	Ripe fruits	20–93	[38]
		C. sativa	Fruits	19	[66]
		C. sativa	Bulk fruit samples	0.17–64.11	[45]
	Portugal	*C. sativa*	Fruit rot	6.4	[67]
		C. sativa	Fruit rot	8.0/5.3/5.0 [3]	[20]
	United Kingdom	*C. sativa*	Canker in the shoots		[27]
	Slovenia	*C. sativa* *C. crenata* × *C. sativa*	Fruit rot; canker in branches		[32]
	Spain	*C. sativa*	Nuts and burrs		[46]
		C. sativa × *C. crenata*	Canker in branches		[28]
	Switzerland	*C. sativa*	Fruit rot; asymptomatic in ripe fruits	91	[47]
		C. sativa	Unspecified in abandoned necrotic galls of *D. kuriphilus*	54	[68]
		C. sativa	Canker in galls and shoots; asymptomatic in shoots, wood, bark, and leaves, as well as at the vascular level		[31]
		C. sativa	Abandoned *D. kuriphilus* galls and wood bark cankers	54	[48]

[1] Western Black Sea region. [2] Incidences of the disease in nuts collected from the ground and burrs, respectively. [3] Data for the years 2017, 2018, and 2019.

3. Incidences of the Disease Caused by *G. smithogilvyi*

G. smithogilvyi is already recognized today as one of the most severe threats to chestnut production due to the significant economic losses that this fungus can cause, mainly in post-harvest [21,22,55]. Table 2 compiles the incidence of the disease reported for *G. smithogilvyi*, ranging from 5 to 94%, in Chile and Italy, respectively. Specifically in Europe, the highest infection rates of fruit rot caused by *G. smithogilvyi* were reported in Switzerland and Italy (54 to 91% and 20 to 94%, respectively). Portugal still has a low value, less than 10%. Outside Europe, *G. smithogilvyi* has been reported to cause high levels of infection in Australia (around 72%), followed by India (33–58%) and Chile (5%).

The disease is mainly expressed after fruit harvesting, although there are already reports of its presence in fruits in the pre-harvest phase when fruits are still in the tree [21,38,42,47].

In Portugal, Driss [67] identified the presence of the fungus *G. smithogilvyi* in industrial samples of Portuguese chestnuts, having determined an infection rate of 6.4%. Likewise, Coelho and Gouveia [20] isolated and identified this fungus in commercial chestnut samples in 2017, 2018, and 2019, with a fruit infection ratio of 8.0, 5.3, and 5.0%, respectively. In five chestnut groves in the Trás-os-Montes region (northeast of Portugal), in 2018, the same authors found an incidence of the disease by *G. smithogilvyi* between 0 and 4.3%. Lione et al. [38], in twelve groves sampled in 2011 in northern Italy, found an incidence of the disease ranging from 20 to 94% in the fruits analyzed. Another study, which reports for the first time the presence of *G. smithogilvyi* in Chile, identified an average incidence of the disease of 4.8% (range equal to 3.7–6.5%) in 31,851 fruits surveyed from the market during 2018, 2019, and 2020 [23]. In Northern India (Kashmir), an incidence of the disease by *G. smithogilvyi* ranged from 33 to 58% in chestnuts harvested randomly from 2009 to 2013 [26]. The differences reported for the countries can be related to the climate [38], the occurrence of drought periods, and the presence of *D. kuriphilus*, which are stress factors for the chestnut trees [42] and may induce higher incidences of the disease. In fact, higher temperatures are associated with higher incidences of the disease [38]. Furthermore, it seems that areas that are more isolated may be a barrier to the settlement of this fungus [26], decreasing the disease incidence.

Therefore, these results demonstrate the possibility of high percentages of *G. smithogilvyi* infection in the near future, which will cause significant economic losses. In fact, producers are paid, taking into account the quality of the product supplied to the industry. The product is randomly sampled when it is delivered to the manufacturing facilities. Therefore, fruits with a high rot rate will entail a lower price paid to the producer, which has associated costs (request for labour, rental of harvesting machines, fuel, etc.) that may not be fully supported with the product sale. Thus, this activity can be abandoned due to a lack of profitability.

4. Damages Caused by *G. smithogilvyi*

Infection with the fungus *G. smithogilvyi* is responsible for the degradation of texture, dehydration, and alteration of the interior colour of the fruits, causing the loss of quality and commercial value of this nut [18,22,43].

The symptoms caused by the pathogenic fungus *G. smithogilvyi* have been reported mainly in Australia, Italy, and France. These include pre- and mostly post-harvest fruit rot, canker in various plant tissues (e.g., shoots and branches), and necrosis in leaves, as well as in galls caused by *D. kuriphilus* (Table 2).

Although the fungus *G. smithogilvyi* is already considered a danger to the production and transformation of chestnut, its presence is not yet widely disseminated in Portugal. Thus, it is essential to study this fungus to alert producers and find effective solutions for its control as soon as possible.

At the production level, producers have also faced the problem of the chestnut gall wasp (*D. kuriphilus* Yasumatsu) that causes severe damage to chestnut cultivation worldwide [17]. This insect attacks plants of the genus *Castanea*, inducing the formation

of galls in the buds and leaves. These galls can reduce tree growth and nut production, leading to a significant reduction in fruit production and quality [69,70]. As previously reported, *G. smithogilvyi* has been observed in association with galls induced by *D. kuriphilus* [25,27,43,45,60,62,64,65,71]. In Spain, Fernández et al. [71] isolated *G. smithogilvyi* from necrotic galls, being the fungi with the highest frequency, closely followed by *Fusarium avenaceum*. However, there are contradictory results regarding the type of association *G. smithogilvyi* and chestnut gall wasp established, pointing in some cases to synergism [44] while in others to antagonism [43]. For example, Lione et al. [44] observed that the number of emerging adults of *D. kuriphilus* was significantly higher in galls colonized with *G. smithogilvyi*, suggesting a possible synergy between the pathogen and the pest. Vinale et al. [61] observed that *G. smithogilvyi* produced abscisic acid (ABA) and 1′,4′-*trans*-diol ABA inside necrotic galls. ABA has been considered a negative regulator of disease resistance [62]. Therefore, the colonisation of the pathogen in oviposited buds would be favoured. In contrast, Vannini et al. [43] observed an increase in the compaction and hardness of the necrotic galls due to the infection of *G. smithogilvyi*, which prevented the exit of the *D. kuriphilus* adults, who remained trapped. Thus, these results show the need to conduct further studies on this topic.

5. Strategies to Mitigate the Damage Caused by Moulds and, in Particular, *G. smithogilvyi*

Some works have been carried out at both the pre-harvest and post-harvest levels of the European chestnut to increase the shelf life of the fruits. Table 3 compiles the studies carried out so far on this topic.

Table 3. Methods to prevent and control *G. smithogilvyi* during pre-harvest and post-harvest.

Stage	Method	Reference
Pre-harvest	Preventive - Removal of litter following harvest	[18]
	Control	
	- Use of antagonists, such as *Bacillus amyloliquefaciens* and *Trichoderma atroviride*	[72,73]
	- Use of fertilizers, such as Kalex Zn product (Alba Milagro®), and application of Mystic® 430 SC (Nufarm Italia Ltd., Milano, Italy, at 40.18% (*w*/*v*) of tebuconazole. This product is used as conventional chemical treatment against fungal contamination)	[74]
	- Use of fungicides [1]: pyraclostrobin; prochloraz; iprodione; fludioxonil; difenoconazole; cyprodinil + fludioxonil; pyraclostrobin + difenoconazole	[75]
Post-harvest	- Disinfectants [2]: hydrogen peroxide + peracetic acid; trifloxystrobin; hydrogen peroxide + peracetic acid + caprylic acid + glycolic acid + capric acid; fludioxonil; caprylic acid + glycolic acid + capric acid; chlorine dioxide; sodium metabisulfite; sodium hypochlorite; aluminum hydroxide acetate; copper sulphate; aqueous ozone; peracetic acid.	[76]
	- Post-processing treatments applied to inoculated peeled chestnuts [3]: X-ray irradiation (70 kV/57 mA, doses 0.5, 1, 1.5, 2 kGy); StorOx (2700 ppm hydrogen dioxide + 200 ppm peracetic acid); Agri-cide (1 ppm); 10 ppm chlorine dioxide solution; 0.70 ppm ozone solution; 80 ppm peracetic acid; 100 ppm chlorine solution; 0.2 M sodium chloride; warm water at 65 °C)	[77]
	- Gaseous ozone	[66]
	- Hot-water treatment + enzymes able to degrade the fungal cell wall	[63]

[1] Active compounds. [2] Concerning the mould severity on the shell and the incidence of decay in the kernel. [3] Concerning mesophilic aerobic bacteria and yeast counts. Aprox. 500 g of inoculated chestnuts were immersed in each solution for 2 min. Active agents: StorOx (27% hydrogen dioxide + 2% peracetic acid); Agri-cide (18.25–21.75% copper sulfate pentahydrate); chlorine solution (6% sodium hypochlorite).

Applying good management practices in the orchards is essential for reducing the spread and inoculum density of *G. smithogilvyi*. Dobry and Campbell [18] suggest removing the litter after harvesting to reduce the inoculum. Nevertheless, as stated by the authors, more studies need to be carried out to show a positive relationship between the presence of litter and the incidence of the disease.

At the pre-harvest level, Pasche et al. [72] found that both *Bacillus amyloliquefaciens* and *Trichoderma atroviride* are promising biocontrol agents of *G. smithogilvyi*. In fact, the inoculation of the chestnut incisions of the cultivar Monti Cimini of Italy with suspensions of *B. amyloliquefaciens* or *T. atroviride* before grafting inhibited the growth of *G. smithogilvyi* in the bark tissues [72]. The same authors also observed a reduction in the symptoms of bark canker when these antagonists were applied [72]. It was now found that *Trichoderma* spp. isolates were able to suppress *G. smithogilvyi* growth through the production of volatile compounds (VOCs) and non-volatile compounds (nVOCs) [73]. On the other hand, *Bacillus subtilis* was also able to inhibit the fungus growth; however, its main effect was through nVOCs [73].

Bastianelli et al. [74] reports that the application of 300 mL/hL of Kalex Zn (crown spray) during blooming and burr formation efficiently controlled the disease. This product is a fertilizer of Alba Milagro®, composed of zinc phosphonate, potassium phosphite, and phosphonic acid, with a low environmental impact. However, since July 2022, all phosphonates were eliminated from the European fertilizers database as indicated in Regulation (EU) 2019/1009 of the European Parliament and the Council of 5 June 2019 [78]. However, new guidelines are expected regarding the application of these products in the field, considering their role in the orchard, and for which products they can be applied. Good results were also reported after the application of the fungicide Tebuconazole [74] (Mystic® 430 SC from Nufarm Italia Ltd., Milano, Italy). Silva-Campos et al. [75] studied the effectiveness of several fungicides by in vitro assays. Moreover, they verified that the most effective were those based on pyraclostrobin and difenoconazole, both effective in suppressing the conidial germination and mycelial growth of *G. smithogilvyi*. In the field, the treatment with both compounds simultaneously reduced the percentage of infected nuts. However, the use of this mixture should be used with care. It must be performed in rotation with other fungicides to avoid inducing resistance in *G. smithogilvyi* or other fungal populations [75].

Regarding post-harvest, the number of studies carried out until now to control the infections caused by *G. smithogilvyi* is also very scarce. Moreover, most of these studies focused on the control of the total microbial and fungal loads, with the species responsible for the symptoms on fruits not being specified. According to Donis-González et al. [77], fungi are responsible for significant economic losses in the post-harvest period. Fruit contamination takes place before and at the time of harvest, increasing during transport and storage. These authors, when studying the effect of several chemical disinfectants to mitigate the damage caused by moulds, found that the use of 2700 ppm of hydrogen peroxide + 200 ppm of peracetic acid (StorOx®), and 0.15 ppm of trifloxistrobin (Flint®) significantly reduced the presence of moulds in the bark and the incidence of the rot of the kernel. Among the treatments studied by Donis-González et al. [76], the combined use of chemical disinfectants with good agricultural practices and good post-harvest management was shown to be the most efficient for protecting chestnuts against the development of moulds and the appearance of rot in the post-harvest period. Moreover, Donis-González et al. [77], when studying the microbial contamination in peeled chestnuts, verified that the bacteria *Rahnella* spp. and *Curtobacterium* spp. and yeast *Candida* spp. were always isolated from vacuum-packaged samples, the methods with 2700 ppm hydrogen dioxide + 200 ppm peracetic acid, warm water at 65 °C, and X-ray irradiation being the most effective in reducing microbial counts. Nevertheless, some concerns arise when using chemical substances in high quantities because they may cause human health and environmental impacts. As stated by Toolkiattiwong et al. [79], even though the application of some pesticides may not exceed the recommended dose, the monitoring of some substances

should be performed. This situation will depend on the chemical properties of the substance and its long-term health effects [79]. Furthermore, the use and the management of fertilizers must be improved because they are essential for reducing the crop water scarcity footprint and the water eutrophication footprint by decreasing the nitrogen leaching into waters [80].

Ozone application may be a good alternative to chemical disinfectants. Vettraino et al. [66] verified that ozone is an appropriate and economical tool to maximize the quality of chestnut shelf life, enabling it to be stored for long periods. Nevertheless, further studies on the ozone-based treatment of fruits must be carried out. In industry, the chestnuts are generally subjected to a hot-water treatment, called "curatura", with the main objective of killing the larvae of pests, mainly *Curculio* spp. and *Cydia* spp. However, the addition of enzymes to this water, with the ability to lysate fungal cell walls, has been tested to control moulds in chestnuts during storage. For example, Ruocco et al. [63] showed that the addition of a mixture of enzymes obtained from the fungus *Trichoderma harzianum* Rifai strain T22 to the treatment with hot water (50 °C, 45 min) significantly reduced the incidence of rot caused by *G. smithogilvyi*. Additionally, they found that in chestnuts subject to this treatment, after two months of storage at room temperature, 50% remained in good condition, as opposed to 15% in untreated fruits.

Morales-Rodriguez et al. [81], when using hot water at different temperatures for the treatment of chestnut in the post-harvest period, found that the application of a temperature of 50 °C for a time of 30 or 45 min completely inactivated *G. smithogilvyi*, regardless of the level of colonization and stage of rot. However, this treatment did not reduce the impact of other moulds responsible for the chestnut's external contamination and mycotoxin production. However, the same authors [81] observed that the increase in temperature and time of the treatment caused significant losses in fruit quality. In Portugal, all the chestnut fruits for exportation must be subjected to a hot-water treatment of at least 48 °C for 45 min in a continuous system (and 50 °C for 45 min in the case of exports to Canada) [82]. This treatment aims to eliminate insects or live larvae of *Cydia pomonella*, *Cydia flagiglandana*, *Cydia splendana,* and *Curculio elephas*. However, this process requires a large consumption of water and energy, and its efficacy in the inactivation of *G. smithogilvyi* is unknown.

In general, these methods show the importance of applying the correct pre- and post-harvest methods, and both must be considered and carried out together. On the one hand, it is essential to reduce the microbial load and dispersion in the orchards. Therefore, the application of agricultural practices and the addition of fertilizers and fungicides must be adequately performed. However, the possible creation of resistance to fungicides must be considered and always avoided. On the other hand, concerning the post-harvest methods, it is crucial to study the effect of common disinfectants, ozone, and hot-water treatment (with and without enzymes) in controlling *G. smithogilvyi* because the role and impact of these methodologies on this fungus are not well-known.

6. Future Prospects

Based on the literature review, it was found that more studies need to be performed, namely in:

- the epidemiology of the disease caused by *G. smithogilvyi*, particularly the elucidation of the factors that determine the development of the disease. Indeed, it is crucial to understand the role played by biotic (pests, pathogens, etc.) and abiotic (climate change, chestnut grove management, etc.) factors on the transition of the endophyte lifestyle to the active virulent pathogen;
- the geographic distribution and frequency of *G. smithogilvyi*. The presence of this fungus has already been reported in various regions of the world, but further studies should be carried out in other countries that are important producers of chestnuts, to better know its distribution in the world;
- the relationships of *G. smithogilvyi* with other fungi, bacteria, cankers, and necrosis of the chestnut tissues. It is essential to find antagonists to this fungus and better

understand the metabolic processes that originate cankers and necrosis in leaves and branches. Furthermore, it is necessary to understand the mechanisms that cause the shift from the endophytic to the pathogenic phase;

- the relationship between the wasp *D. kuriphilus* and *G. smithogilvyi*, in order to better understand the role played by this insect on the incidence of the disease;
- the identification of methods and/or tools to manage the disease caused by *G. smithogilvyi.* These may include the identification of *C. sativa* cultivars that are resistant or at least more tolerant to *G. smithogilvyi* or improve host plant resistance to the pathogen through breeding programs. This information will be of great interest to producers to help them choose the plant material to use in new plantations as well as to prevent the disease's development in new plantations. A potential additional measure includes the identification of biocontrol agents, such as micro-organisms, with efficacy against *G. smithogilvyi*. Furthermore, several cultural practices, such as the removal of fallen burrs (in which the teleomorph stage of the fungus develops) as well as of severely infected plant residues in the field, may all have important implications on the spread of *G. smithogilvyi* and disease management;
- the identification of the means of spread of the pathogen, either at short or long distances. Indeed, *G. smithogilvyi* could potentially spread at short distances via natural means (e.g., wind, water splash, and insects) and/or at long distances via human-assisted means (e.g. movement of infected host plants for planting), similarly to other pathogenic fungi;
- the development of methods for the early detection *G. smithogilvyi* in the groves;
- the elucidation of the mycotoxigenic potential of *G. smithogilvyi*. This work is extremely important, since its potential is not known;
- the identification of post-harvest management methods for reducing *G. smithogilvyi* growth, such as the use of disinfectants.

7. Conclusions

There is still a long way to obtain more knowledge on *G. smithogilvyi*.

It is important to: (i) understand its biological cycle; (ii) elucidate the infection processes that cause canker and necrosis in leaves and branches; (iii) know its role on the galls of *D. kuriphilus*; (iv) understand the mechanisms that trigger the change from the endophytic to pathogenic phase; (v) understand what ecological factors can be the precursors of outbreaks of this fungus; and (vi) find post-harvest technologies to manage this pathogen, such as the use of disinfectants, ozone, and hot-water treatment (with and without enzymes) in controlling *G. smithogilvyi*.

This fungus is able to depreciate the quality of chestnut very significantly and affects its production. Thus, all potential biotic interactions must be analyzed. In addition, the precautionary principle should be considered when considering the risk associated with this pathogen on a global or local scale.

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