SURVEY AND GEOGRAPHIC DISTRIBUTION OF CHESTNUT BLIGHT IN PORTUGAL

PROSPECÇÃO E DISTRIBUIÇÃO GEOGRÁFICA DO CANCRO DO CASTANHEIRO EM PORTUGAL

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ABSTRACT

Cryphonectria parasitica, the fungus that causes chestnut blight disease, was first described in Portugal (1929) on bark samples of *Castanea crenata*, collected in Beira Interior province. After this report, only in 1989 two disease foci were found on native European chestnut, *C. sativa*, in Trás-os-Montes e Alto Douro Province. To assess the current distribution of chestnut blight in Portugal (mainland, Azores and Madeira) a nationwide monitoring program was implemented. A major cooperative effort, involving all the Portuguese Forest and Agricultural Services, was created for this endeavor and coordinated by the National Forestry Station. A total of 191 chestnut stands was surveyed across the entire country. *C. parasitica* was isolated from 56.5% of the sampling sites (108 sites). Sampled parcels were digitally geo-referenced and mapped. In addition, a collection of *C. parasitica* isolates has been established and the study of the molecular variability of *C. parasitica* in Portugal is underway.

Key-words: *Cryphonectria parasitica, Castanea sativa,* Portugal

RESUMO

Cryphonectria parasitica, o fungo responsável pelo cancro do castanheiro, foi descrito pela primeira vez em Portugal (1929) em amostras da casca de *Castanea crenata*, provenientes da Beira Interior. Depois da detecção inicial, apenas em 1989, dois focos da doença foram encontrados em castanheiro Europeu, *C. sativa*, na província de Trás-os-Montes e Alto Douro. Para determinar a distribuição actual do cancro do castanheiro em Portugal (continente e Regiões Autónomas da Madeira e Açores), foi implementado um programa nacional de monitorização da doença. Para o efeito houve intensa cooperação entre os Serviços Florestais e Agrícolas do país, com coordenação da Estação Florestal Nacional. Foram visitadas, em todo o país, 191 parcelas de castanheiro. *C. parasitica* foi isolado em 56,5% da amostragem (108 parcelas). As parcelas amostradas foram digitalmente georeferenciadas e cartografadas. Uma colecção de isolados de *C. parasitica* foi estabelecida encontrando-se em curso o estudo da variabilidade genética da doença em Portugal.

Palavras-chave: *Cryphonectria parasitica, Castanea sativa,* Portugal

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INTRODUCTION

In Portugal, *Cryphonectria parasitica* (Murrill) Barr was first described by Câmara (1929), on bark samples collected on branches from young plants of *Castanea crenata* Sieb. & Zucc, in the Beira Interior Province, near the village of Alcaide, Fundão. Forest Services Publications reported in 1949 that "no focus of chestnut blight was found in Portugal until now, although we are not certain that it does not exist since many Japanese chestnuts have been imported in the last years" (Fernandes, 1949). The initial infection was probably successfully eradicated, since only in 1989 two disease foci were found on European chestnut (*Castanea sativa* Mill.) in the Trás-os-Montes e Alto Douro province, the most important portuguese chestnut-growing region in the Northeast of the country, in Carrazedo de Montenegro (Serra da Padrela -Valpaços) and Parada village, Terra Fria – Bragança, (Abreu, 1992).

After the 4th meeting of the International Commission for Chestnut, in 1958, Yugoslavia, Portugal is included within the range of the fungus and in the geographic distribution of the disease in the Commonwealth Mycological Institute (CMI) records (Caetano, 1990). In 1990 the disease was of mandatory report by governmental ordinance ("Portaria 847/90, 18 Setembro"). In spite of this fact and in opposition to the general rule giving priority to eradicate critical quarantine diseases, no financial support was allocated for this purpose. This measure was only taken in 1998 ("Despacho Conjunto 117/98, 18 Fevereiro") in compliance with the establishment of an eradication program which included logging and incineration of diseased branches or the entire tree (Anastácio, 2001).

During the three years of the program, a total of 82 000 chestnut trees were monitored in Northern Portugal by the Direcção Regional de Trás-os-Montes e Alto Douro services (DRATM). The overall rate of infection at that time was approximately 10%, although major differences among localities were observed. However, the disease was present in all counties. In the Chestnut Protected Denomination of Origin zone (PDO) of "Castanha da Padrela", one of the most important in the country, 61 528 trees were monitored with an infection rate of 100% reported in some localities (Anastácio, 2001).

In subsequent years, the disease spread by natural pathways and/or by the influence of man, intensifying chestnut monoculture due to increasing economical interests and investments in chestnut orchards and forest stands (Abreu & Martins, 2002). Overall, the balance of the program was that the eradication objectives were not totally achieved. Although the implementation of eradication measures allowed the decrease of the levels of infection. In addition, the improvement of good cultural practices by the growers, also implemented during the program, was an important factor preventing the fast spread of the disease.

The Portuguese archipelago of Azores ("Região Autónoma dos Açores") reported chestnut blight for the first time in 1993. Two internal reports from S. Miguel and Terceira Islands made by the Estação de Fruticultura Vieira Natividade in Portugal and Institute Nationale de Recherche Agronomique (INRA) in France, described the occurrence of the disease. In addition, Ormonde (1994) describes that symptomatic trees in the Terceira Island were not significantly affected by the disease, thus, hypothetically the hypovirulence could also exist at that time and later Abreu and Martins (2002) referred the existence of an hypovirulent culture from Azores. That situation was also observed *a posteriori* and referred by Bragança et al (2004). In Madeira Island ("Região Autónoma da Madeira"), chestnut blight was detected for the first time in 2002 by the Direcção Regional de Agricultura da Madeira authorities (DRAM), in chestnut stands of "Comissão de Levadas do Curral e Castelejo", Curral das Freiras village. Laboratorial confirmation of the disease was performed in the Laboratório Agrícola da Madeira (Maia, 2003).

To assess the distribution of chestnut blight and the variability of *C. parasitica* in Portugal,

it was crucial to put into action a nationwide monitoring program comprising all chestnut growing regions in Portugal. The experimental design of this work would have to assure both the accurate estimate of the distribution of chestnut blight, which previously was presumed to be centralized only in the Northeast of the country, and the characterization of all the existent populations of the fungus. The study of the genetic diversity in populations of *C. parasitica* and the research for hypovirulent isolates were required to implement, in the nearest future, effective actions preventing further spread of the disease.

In the aim of this work all chestnut growing regions in Portugal were visited and a national collection of *C.parasitica* isolates has been established. Considering the importance of the chestnut area and the incidence of chestnut blight in Trás-os-Montes e Alto Douro, some areas in this province were most intensively prospected. In the course of the survey it was possible to observe signs and symptoms not only of *C. parasitica* but of other chestnut diseases as well. Although not a primary goal of this study, additional information on other fungi found in chestnut stands is also briefly presented.

MATERIAL AND METHODS

Experimental design

The selection of the sites to be monitored for the presence of chestnut blight sought to cover all the area of chestnut in Portugal with the inclusion of the possible highest number of sites previously known to present the disease.

Sample sites were selected considering the information given by the Agriculture and Forest Services and the map of chestnut distribution area in the country. All provinces in continental Portugal, Madeira Island, and three Islands of the Azores were surveyed for chestnut blight in the course of the monitoring program.

In each of the regions, assessment of all chestnut sites (by district parish – "freguesia") with an area >0.5 ha was done. Subsequently, these areas were monitored for the presence of *C. parasitica*. In each district parish, as a rule, sites with previously reported presence of chestnut blight were always monitored. For the remaining selected areas, monitoring sites were randomly selected and then inspected for the presence of symptoms and/or signs of the blight.

Cartographic localization of sampling areas was performed using military cartography and orthophotomaps (paper based and, when available, digital materials). Sampling sites and localities were digitally geo-referenced and mapped using GIS analysis tools through ArcView® 3.2 software (Environmental Systems Research Institute, Inc.). The number of sites monitored per region, as well as, the number of *C. parasitica* isolates per site, varied largely according to the distribution of chestnut in Portugal and the incidence of chestnut blight, respectively. Distinct data from the sites (*e.g.* signs and symptoms of disease, stand characterization, environmental conditions, etc.) and individual tree descriptions were gathered in order to find correlations with the disease.

Field procedures involved visual assessment of all the trees inside the sites in order to detect chestnut blight symptoms: branch or tree mortality with shrivelled, dried leaves, chestnut blight cankers with longitudinal bark fissures and presence of fungal stromata or mycelial fans. Based on Robin et al. (2000) each sampling site was approx. 1 ha in size and one canker per tree was sampled for a maximum of ten different trees per site (sporadically, in larger sites more than ten trees were sampled).

Isolation and identification

Using a knife, bark samples (approx. 2 x 2 cm) were removed from the cankers and brought to the laboratory. Small pieces (approx. 2 x 2 mm) were cut out from the bark samples and surface-disinfected by dipping for 15s in 70% ethanol. Then, the samples were rinsed in sterile water, blotted on filter paper and placed on potato dextrose agar

(PDA, Difco, Sparks, MD, USA). The plates were incubated at 25ºC in the dark and C. *parasitica* cultures were transferred to new PDA plates. One isolate per canker was used for further analysis.

Long-term storage of *C. parasitica* isolates

Before stocking, the isolates were grown on PDA medium placed in Petri dishes and McCartney bottles, then stored at 25ºC in 24h dark for seven days. Subsequently both containers were transferred to a laboratory bench with natural light during 10 to 14 days to allow profuse production of conidia. *C. parasitica* isolates were stored at 6ºC on McCartney bottles (three bottles per isolate). In parallel, the isolates were also frozen in glycerol and stored at -80ºC as follows: 3-4 pieces of PDA (5x5mm) were cut from sporulating cultures and immersed in 800µL of a 22% sterile glycerol solution (Riedel-deHaën, Seelze-Hannover, Germany) allocated in Cryo-vials tubes (Tenak™), mixed heavily by vortexing and finally frozen in liquid nitrogen before storage.

Detection of other phytopathogens

Chestnut sample tissue with signs of other diseases was observed and if necessary processed in laboratory through slide preparations for microscopic observation and posterior confirmation of the phytopathogenic agent of genus level. In some cases, soil analyses were performed in the forest pathology laboratory to confirm the presence of *Phytophthora* sp. as described by Campbell (1949).

RESULTS

A total of 191 chestnut stands were monitored across the entire country comprising 55 district parishes in 34 counties from all Regional Agricultural Department areas in Portugal mainland, and 25 district parishes from 13 municipality areas in the Archipelagoes). (Fig. 1, Table 1)**.** The results obtained herein, and presented in Table 1 and Figs. 1 to 7 indicate that chestnut blight has spread into most of the chestnut growing area of Portugal. Signs and symptoms of the disease have been detected in six Regional Agricultural Department areas of the mainland and *C. parasitica* was isolated from four of these six regions. The disease was also found in the Azores and Madeira archipelagoes. A total of 617 *C. parasitica* isolates were obtained from 108 sampling sites (mainly orchards) distributed across four mainland provinces (Minho, Trás-Montes, Beira Interior, Alentejo), the Azores Islands (Pico, Terceira, S. Miguel), and the Madeira Island.

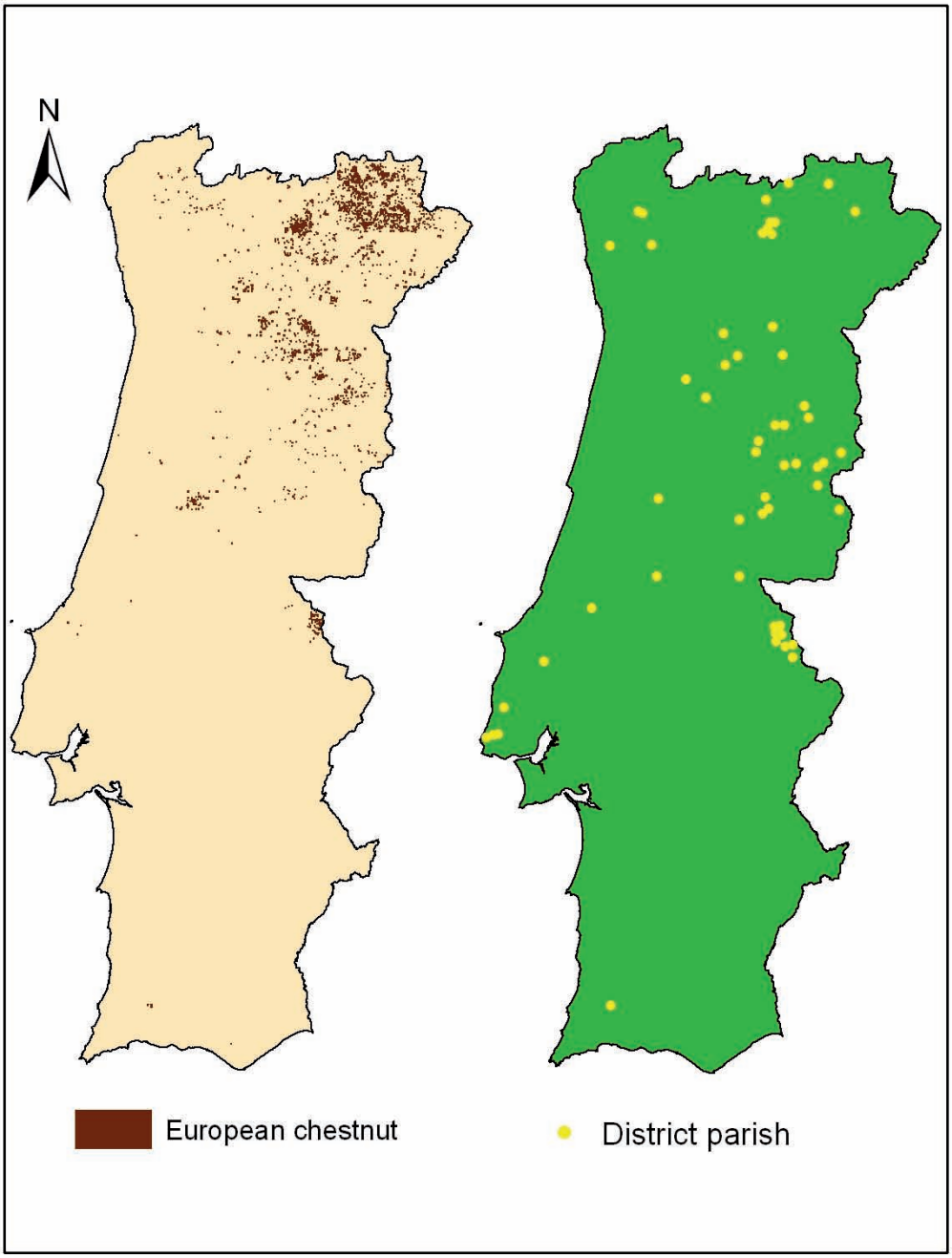

Figure 1 – Portugal mainland: distribution of European chestnut tree and chestnut blight survey by district parish.

Table 1 – Survey and distribution of chestnut blight disease in Portugal.

	Regional Agriculture Department in mainland/ Islands	Counties "concelhos"	No. of Sites inspected	No. of Sites with *C. parasitica* isolates
Portugal (mainland)	**DRAEDM**	Vila Verde Amares Barcelos Guimarães	**4 (forest stands)**	**4**
	DRATM	Chaves Valpaços Vinhais Bragança Tarouca Penedono	**73**	**58**
	DRABI	Trancoso Guarda Manteigas Belmonte Sabugal Fundão Penamacor Castelo- Branco Idanha-a-Nova Vila Velha de Rodão	**23 (14 forest stands)**	**6**
	DRABL	Castanheira de Pêra Lousã Vila Nova de Paiva S. Pedro do Sul Viseu	**6 (2 forest stands)**	**0**
	DRARO	Alcanena Ferreira do Zêzere Cadaval Mafra Sintra*	**10 (8 forest stands)**	**0**
	DRAAL	Portalegre Castelo de Vide Marvão	**25 (2 forest stands)**	**7**
	DRAALG	Monchique*	**2**	**0**
Azores ("Região Autónoma dos Açores")	**PICO**	Madalena S. Roque do Pico	**6**	**2**
	TERCEIRA	Angra do Heroísmo Vila Praia da Vitória	**14**	**12**
	S. MIGUEL	Ponta Delgada Ribeira Grande Lagoa Vila Franca do Campo Nordeste Povoação	**18**	**16**
Madeira ("Região Autónoma da Madeira")	**MADEIRA**	Ribeira Brava Câmara de Lobos Funchal	**11**	**3**

* Includes sites with signs and symptoms of chestnut blight.

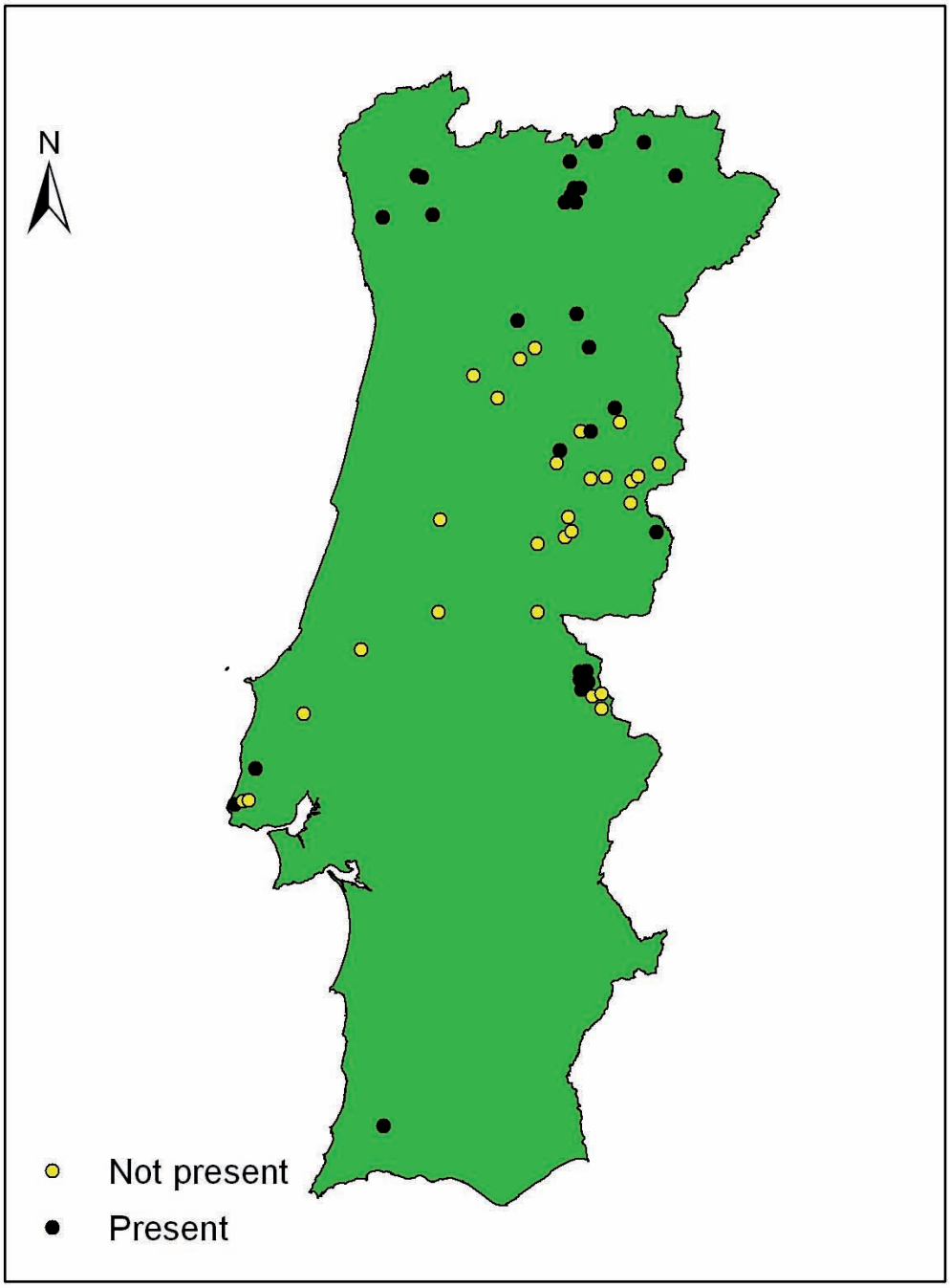

Figure 2 – Geographic distribution of chestnut blight in Portugal mainland (by district parish).

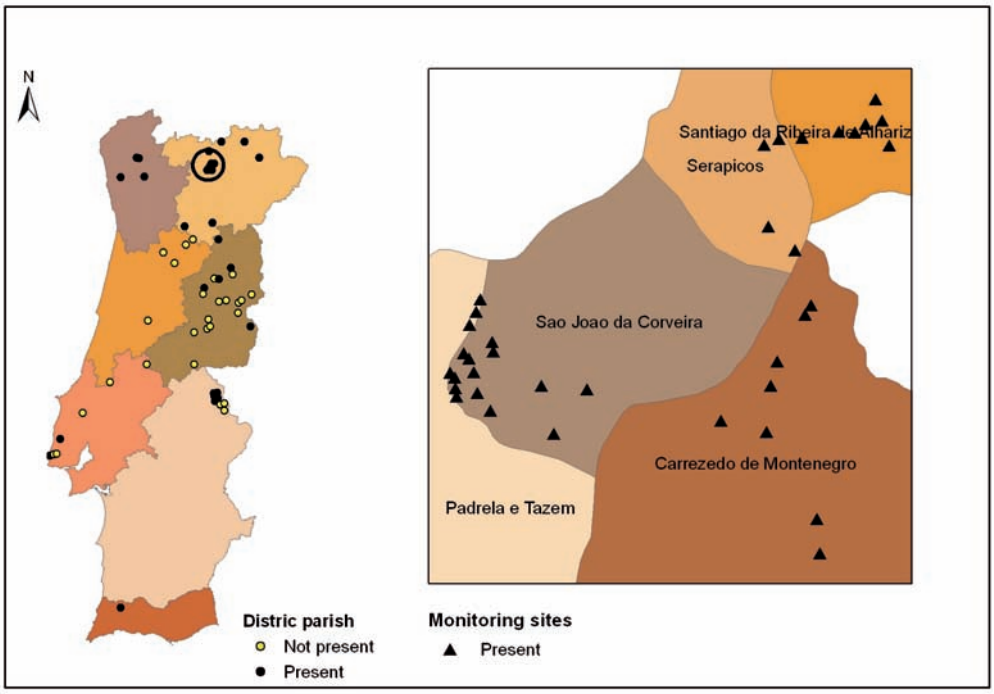

Figure 3 – Geographic distribution of chestnut blight - Valpaços detail (in Trás-os-Montes e Alto Douro).

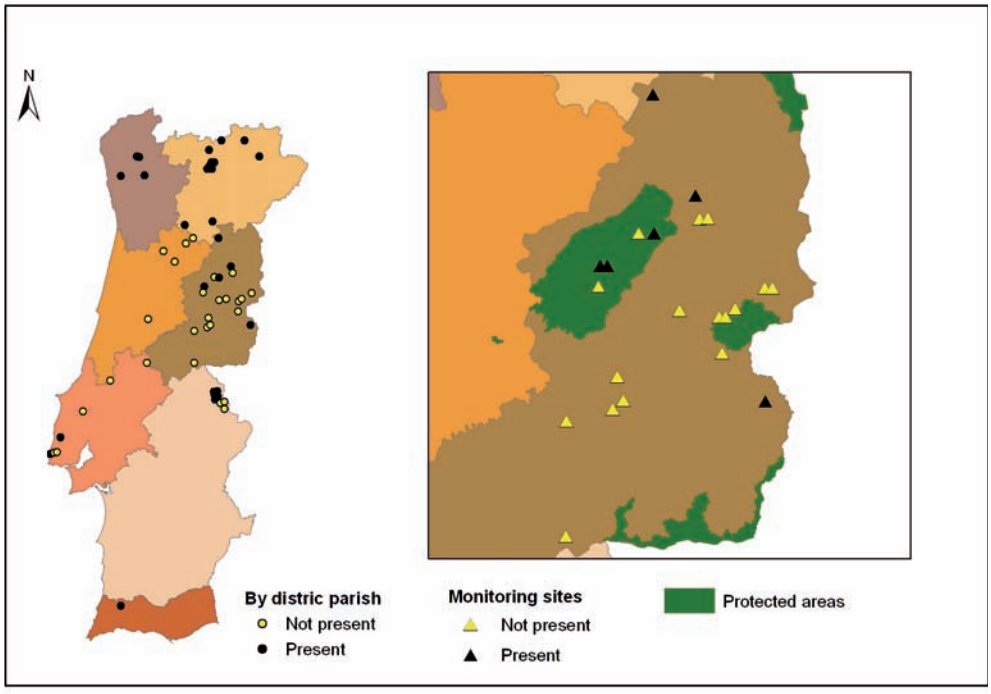

Figure 4 – Geographic distribution of chestnut blight - Beira Interior detail.

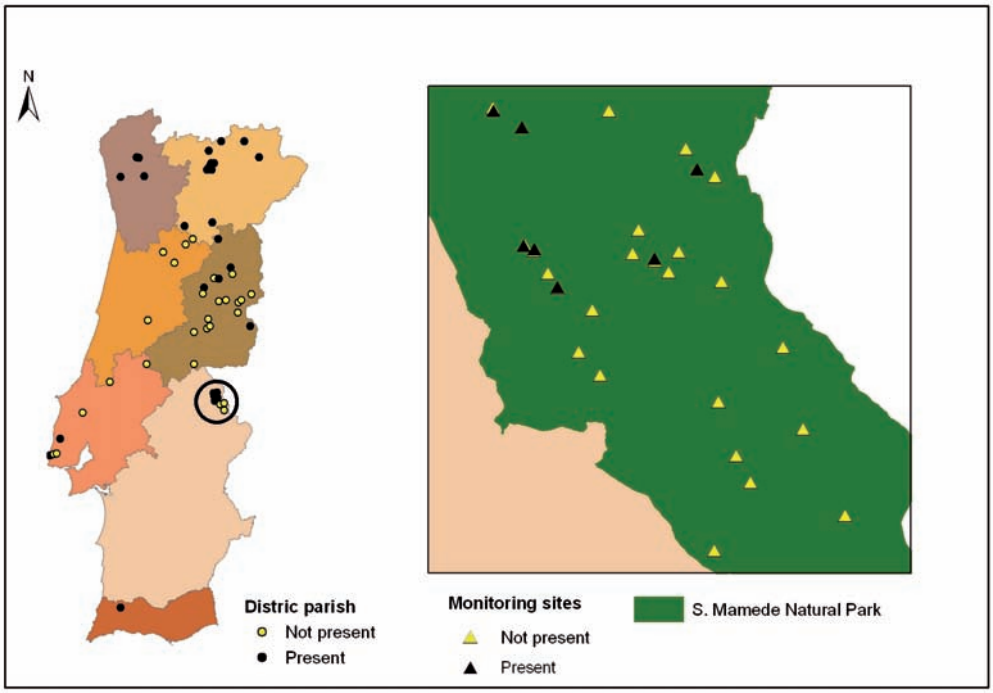

Figure 5 – Geographic distribution of chestnut blight - Alentejo detail.

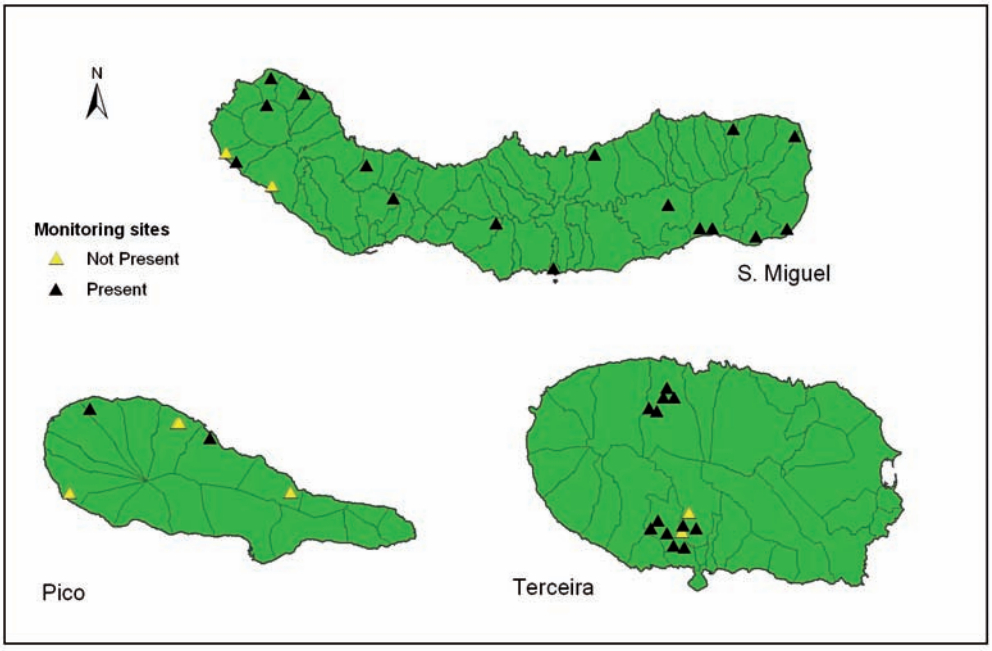

Figure 6 – Geographic distribution of chestnut blight – Azores Islands detail (lines inside the maps represent District parish borders).

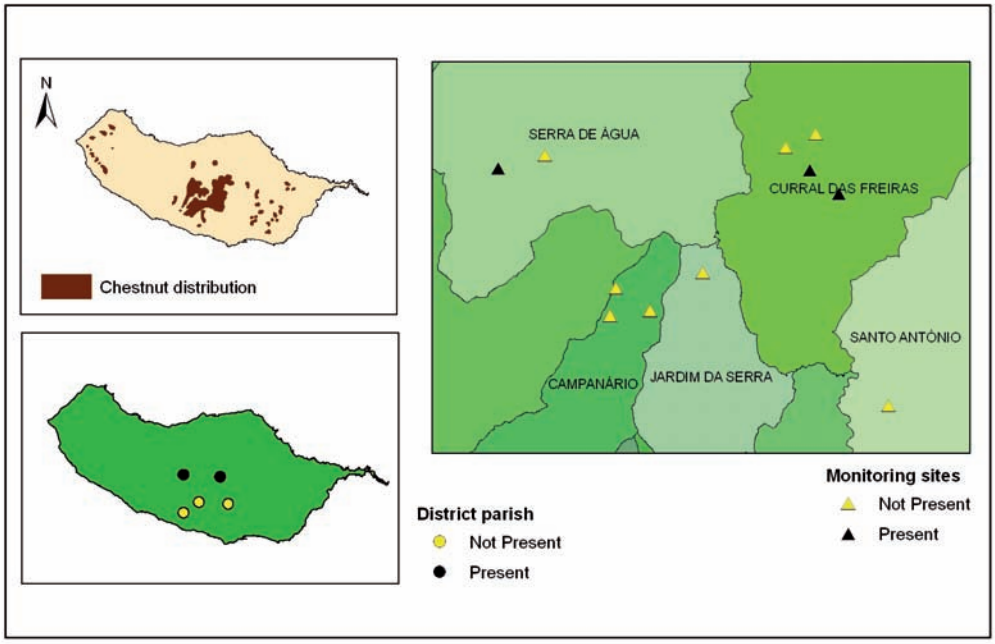

Figure 7 – distribution of chestnut blight – Madeira Island detail.

Regarding the survey of the other diseases, the following phytopathogenic fungi were identified: *Coryneum* sp., *Cytospora* sp. and *Phytophthora* sp.. Symptoms of ink disease were observed in all visited regions, diffuse cankers caused by *Coryneum* sp. were observed mainly in Trás-os-Montes e Alto Douro region and *Cytospora* sp. was found affecting young plants in São Mamede Natural Park in Alentejo region.

DISCUSSION AND CONCLUSIONS

Portuguese chestnut stands monitored during this work varied in structure and composition. In Trás-os-Montes e Alto Douro, the most important chestnut area in Portugal, most chestnut areas form a continuous surface of orchard stands. In Beira Interior, the type of chestnut stand is heterogeneous, with both small and extended orchards, high forest and coppice areas. Overall, the high forest and coppice stands where the disease was observed were known to have suffered past human interventions, although at a much smaller rate than the orchards. Conversely, the vast majority of the forests without conspicuous human intervention and management were found to be disease-free, with the exception of only two places, both in Beira Interior province. Furthermore, important portuguese ecological hotspots were also sampled during this survey, i.e., the Serra de São Mamede Natural Park and Serra da Estrela Natural Park. These zones represent vulnerable ecosystems, which constitute a valuable ecological patrimony in Portugal. Chestnut stands in both areas were found to be extensively affected by chestnut blight.

The results reported here constitute a pioneer and baseline work from which several other studies have been incorporated aiming to: 1) Determine the diversity of vegetative compatibility types and mating types of *C. parasitica* in Portugal, as well as, the incidence of natural hypovirulence (Bragança *et al.* 2007). This information is required to evaluate the potential for biological control of chestnut blight, following strategies im-

plemented in other regions of the world, and constitutes an essential step in order to block the dissemination of the disease; 2) Analyse the genetic diversity of *C. parasitica* in Portugal mainland and archipelagoes to discern as to the phylogenetic history of *C. parasitica* in the country; 3) Assess the presence of the sexual form of the pathogen, the perithecia, elucidating about its reproductive strategy in infected chestnut areas; 4) Ultimately, to evaluate the biotic and abiotic factors which may be directly or indirectly involved in the incidence of the disease.

Our results suggest that the current distribution and severity of chestnut blight in Portugal is of serious concern and that it is imperative to pursue the development of a holistic management strategy, capable of blocking the spread of the disease into the last non-affected chestnut stands, and to optimize the management of the currently infected areas.

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