Incidence of chestnut blight and diversity of vegetative compatible types of *Cryphonectria parasitica* in Trás-os-Montes (Portugal)

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Abstract
In 1989, the first cases of chestnut blight were recognised in Portugal. Quarantine measures for this A2 quarantine organism (OEPP) were not sufficient to control the disease and a program to eradicate chestnut blight was implemented in 1998.

The distribution and incidence of chestnut blight were surveyed in Bragança and Vinhais, in the Trás-os-Montes region in the north of Portugal where chestnut groves are economically important. The survey found that all communities have blighted chestnut trees except one in Bragança and one other in Vinhais. Bragança has an average of 10.3% blighted chestnut trees and Vinhais 8.0%, but the disease incidence varied greatly among different communities and among sample sites in the same community.

Based on the merging/barrage response, four vegetative compatible types were detected in eight sub-populations of *C. parasitica* studied in the region of Trás-os-Montes (P-1, P-2, P-3, P-5). P-2 is the most frequent and the only type present in five sub-populations of *C. parasitica.* More vc types were found in Vinhais where there was one sub-population with P-1, one with P-3, and one with P-1 and P-2. P-5 was found only once, and it showed a barrage response with all other testers.

Keywords: chestnut blight, *C. parasitica,* vegetative compatible types, Trás-os-Montes, Portugal

1 Introduction

The first cases of chestnut blight (*Cryphonectria parasitica* ([Murr]. Barr) occured in Portugal in 1989. The disease was identified by the agricultural services in the region of Trás-os-Montes (northern Portugal) in chestnut (*Castanea sativa* Mill.) groves of Carrazedo de Montenegro (ABREU 1992). The following year more foci of this disease were found in groves in Vinhais (Valpaço) and Bragança (Parada).

The fungus spreads naturally, which, together with farmers' lack of knowledge of this disease led to new cases appearing throughout the country. The situation greatly worsened and a program to eradicate chestnut blight was officially implemented on a national level in 1998.

83% of the chestnut trees in Portugal are found in the region of Trás-os-Montes. Chestnut trees in this region are ecologically adapted and provide a lucrative economic activity.

The aims of this study were to assess the distribution and incidence of chestnut blight in Bragança and Vinhais, two important chestnut-growing areas in Trás-os-Montes. In addition, the diversity of vegetative compatible types of *C. parasitica* present in Trás-os-Montes was investigated. With these results it will be possible to assess the efficacy of the eradication program and preview other methods to control the continuous expansion of the disease.

2 Materials and methods

From the list of farmers that had registered with their associations or their regional agricultural services in the program of chestnut blight eradication, three names were chosen randomly in each community of Bragança and Vinhais. Altogether 76 farmers were selected. After contacting them, they accompanied us to their chestnut groves to survey the incidence of chestnut blight. Data was

obtained by visual observation of blight symptoms in each individual tree. The location of the infection (stem or branches) and the severity of the disease were also noted (data not presented). Additional information about the age and the number of trees in each grove was obtained from the farmers.

To assess the diversity of vegetative compatible types (vc types) of *C. parasitica* in Trás-os-Montes, eight chestnut groves were chosen, which had a minimum of twenty blighted chestnut trees. They represented extended regions of chestnut groves and were important foci of chestnut blight in the region. In each sample site all blighted chestnut trees were sampled in order to isolate *C. parasitica.* Isolates of each sub-population were obtained from different trees, with the exception of Parada and Valpaço (the initial foci of the disease), where the isolates were obtained from sprouts of trees that had been cut down because of the disease. Bark samples were cut from all cankers of trees or sprouts of blighted chestnuts. Within 24 to 48 hours, all samples were dipped in 70% ethanol and small pieces of bark were placed on potato dextrose agar (PDA, Difco, 39g/L). One isolate per canker was selected and stored on slants of PDA at 4 ºC in the dark. In this study only one isolate of *C. parasitica* per tree was selected for analysis.

Tests for vegetative compatibility were performed by the Swiss method (BISSEGGER *et al.*1997). Vegetative compatibility of the strains was assessed according to the barrage/merging response. Each isolate of a sub-population was paired with all the other isolates of the same sub-population and afterwards paired with the vegetative compatibility Portuguese testers designated as P-1 and P-2. New vegetative compatibility testers were selected from isolates that were incompatible with the former tester strains.

3 Results

3.1 Distribution and incidence of chestnut blight in Bragança and Vinhais

Data from the chestnut blight survey in Bragança and Vinhais are presented in Table 1 and Figure 1. In Bragança 87 sites were studied and 8532 chestnut trees assessed. Of these 882 were found to be diseased. In Vinhais 28 sites with a total of 1187 trees were studied. Of these 96 were diseased. The percentage of blighted chestnut trees in Bragança was 10.33% and in Vinhais 8.08%.

All communities, except one in Bragança (Quitanilha) and one in Vinhais (Edral), were affected by chestnut blight. In some communities over 20% of the trees were infected, whereas in others the infection rate was still low. The disease incidence among different sites varied greatly. In some inspected sites all chestnut trees are blighted, whereas in others there were no diseased plants.

The highest incidence of chestnut blight was found in the South of Bragança and Vinhais, but the spread of the disease is continuous and some new foci appeared during the course of this study (Parâmio).

Chestnut trees of all ages are affected by chestnut blight. In Bragança the disease is higher in adult plants (>20 years) and in Vinhais the disease is most frequent in trees that are 4 to 20 years old. In both regions it is least frequent in young plants (<4 years).

3.2 Diversity of vegetative compatible types of *C. parasitica* in Trás-os-Montes

Four vegetative compatible types were detected among 200 isolates of the eight sub-populations studied (Table 2 and Fig. 2). The vc type P-2 was the most frequent and was the only vc type found present in the five sub-populations that represent an extended area of chestnut groves in Trás-os-Montes. Only two sub-populations had more than one vc type. In Vilar de Lomba the isolates were obtained from young plants recently grafted and in Valpaço only one isolate had a different vc type.

Table 1. Distribution and incidence of chestnut blight in Bragança and Vinhais.

Communities	N° of sites inspected	Chestnut trees sampled	% diseased trees
Bragança	*87*	*8532*	*10.33*
Coelhoso	13	2157	1.8
Paço	7	621	11.0
Parada	17	1840	28.5
Paradinha Nova	3	1059	10.6
Paramio	5	156	6.4
Q^{la} de Lampaças	3	43	14.0
Quitanhilha	5	600	0.0
Rebordãos	9	637	5.0
Salsas	8	146	25.3
S. Pedro Serracenos	2	337	2.1
Sendas	3	308	7.5
Serapicos	12	628	2.8
Vinhais	*28*	*1187*	*8.08*
Agrochão	5	110	6.4
Curopos	2	70	21.5
Edral	1	50	0
Edrosa	5	480	9.4
Ervedosa	3	51	3.9
Espinhoso	3	122	1.7
Reborelo	8	204	7.4
Vilar de Lomba	1	100	10

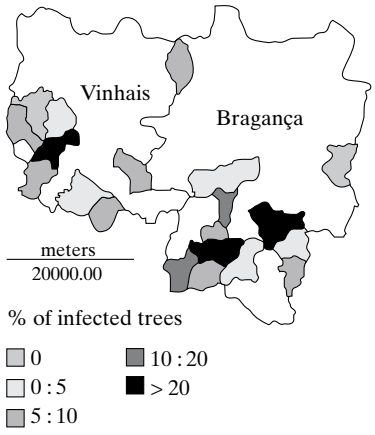

Fig. 1. Spatial distribution and incidence of chestnut blight in Bragança and Vinhais (north-east Portugal).

Table 2. Sub-populations of *C. parasitica,* number of isolates studied and percentage of vc types found.

Sub-population	Seixedo Vila Real	Rio Bom Vila Real	Penedono Guarda	Parada Bragança	Paredes Bragança	Valpaço Vinhais	V. Lomba Vinhais	Curopos Vinhais
Nº Isolates	19	31	22	20	24	24	36	24
vc types (%)	P-2 (100)	P-2 (100)	P-2 (100)	P-2 (100)	P-2 (100)	P-1 (95.8) P-5 (4.2)	P-2 (86.1) P-1 (13.9)	P-3 (100)

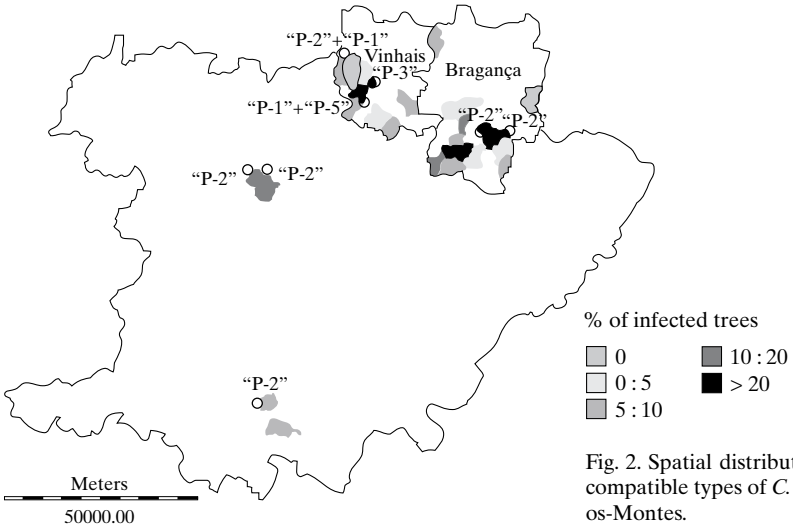

Fig. 2. Spatial distribution of vegetative compatible types of *C. parasitica* in Trás-os-Montes.

Vinhais is the region where more vc types were found. There were one sub-population with P-1 (Valpaço), one with P-3 (Curopos) and one with P-1 and P-2 (V. Lomba). In Valpaço only one isolate showed a barrage response with all other testers and was named P-5 type. Isolates of the south of Portugal were paired with the four known Portuguese testers and an additional vc type was identified and named P-4 (data not shown).

4 Discussion

This survey of chestnut blight in Portugal has shown the rapid development of the disease since its first appearance in 1989. Twelve years were sufficient for chestnut blight to become a widespread disease in northern Portugal. However, the disease incidence varies greatly. Some inspected sites have many diseased trees whereas others in the same area do not have any diseased plants. This also applied to the individual farmers who participated in this study and who have chestnut groves in different places. The survey was done in regions where the chestnut blight is present and the results provide a clear picture of the occurrence of chestnut blight in the region of Trás-os-Montes.

As part of the eradication program of chestnut blight, other areas in north-western Trás-os-Montes were also studied. In some places, all the trees on every farm were inspected (ANASTÁCIO *et al.* 2001). Our results showed the same pattern of distribution in Bragança and Vinhais as in north-western of Trás-os-Montes.

The cultural practices of grafting and pruning play an important role in spreading the disease not only within individual groves but also across long distances in that region.

Healing cankers were not found in this survey but the low diversity of vc types in the *C. parasitica* sub-populations studied should favour a successful implementation of biological control based on hypovirulence.

P-1 and P-2 are incompatible with the EU-1, 2, 5, 6, 12, 13, the most common vc types in Europe (ROBIN and HEINIGER 2001). For a European comparison, EU vc type numbers will have to be assigned to the Portuguese testers.

5% of the isolates showed abnormal morphological characteristics, i.e. slow growth and debilitation. The reasons for this debilitation will be examined in a future investigation of the genetic structure and variability of *C. parasitica* in Portugal. Since this pathogen has only recently been found in this region and is a newly formed population, it could be of particular interest to study its genetic variation and evolution.

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5 References

ABREU, C., 1992: A hipovirulência como forma de luta natural contra o cancro do castanheiro. Revista das Ciências Agrárias, Vol. XV, 1–2: 167–169.

ANASTÁCIO, M.; MESQUITA, M.; PONTEIRA, D., 2001: Programa de erradicação do cancro do castanheiro. Jornadas Transfronteiriças do Castanheiro. Maio, Bragança 11–12.

BISSEGGER, M.; RIGLING, D.; HEINIGER, U., 1997: Population structure and disease development of *Cryphonectria parasitica* in European chestnut forests in the presence of natural hypovirulence. Phytopathology 87: 50–59.

ROBIN, C.; HEINIGER, U., 2001: Chestnut blight in Europe: Diversity of *Cryphonectria parasitica,* hypovirulence and biocontrol. For. Snow Landsc. Res. 76, 3: 361–367.

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