

RESEARCH ARTICLE

A methodology to evaluate disease severity: a case study of chestnut blight in El Bierzo region (northwestern Spain)

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Abstract

Correct knowledge of the incidence and severity of disease is essential for implementation of timely and effective management control strategies. In this article, multiple correspondence analysis (MCA) is applied to evaluate the severity of chestnut blight incited by the ascomyceteous fungus *Cryphonectria parasitica*. This economically important bark disease leads to the loss of an important part of the chestnut production and the progressive death of the tree. A total of 7240 living European chestnut (*Castanea sativa*) trees across 452 plots were surveyed in El Bierzo, NW Spain. For each tree, the main stem and canopy were visually assessed for signs of the pathogen and/or symptoms of the disease and the extent of the disease was classified on a qualitative ordinal scale consisting of six levels. The statistical procedure is useful because it quickly analyses measurable, discrete observations from assessed individuals to provide a disease severity measure related to tree features and disease extension inside the tree. The results indicated that the penetration of the pathogen is located in the lower part of the crown and spreads to the tips of the branches in the upper part of the crown. Thus, our results suggest that man-made wounds, when the tree was grafted or pruned, are the main channel of pathogen penetration in El Bierzo region. Disease severity estimates and incidence data for *C. parasitica* across the region were compared. From the perspective of the management and control of the disease, a disease severity value provides a more accurate indication of the scenario of the disease in a region than presence or absence data alone.

Introduction

Chestnut blight, caused by the fungus *Cryphonectria parasitica* (Murrill) M.E. Barr, is a disease which infects several tree genera (Gryzenhout *et al.*, 2006) although the most economically and ecologically important host is the *Castanea* genus. This disease is very damaging to American (*Castanea dentata* [Marshall] Borkh.) and European (*Castanea sativa* Mill.) chestnuts in North America and Europe, respectively (Hepting, 1974; Heiniger & Rigling, 1994). The pathogen attacks all the aerial parts of the tree: the main stem, branches and young suckers; infecting the tree via wounds in the bark and forming sunken canker due to necrosis and collapse of bark tissue (Hebard *et al.*, 1984; Griffin, 1986). The buff-brown mycelia of the fungus invades and destroys the inner bark (i.e. phloem,

vascular cambium and xylem) resulting in the death of host tissue distal to the point of infection. Thus, the disease leads to the loss of an important part of the chestnut production and the progressive death of the tree. Once established, the bark of the young branches appears reddened and cracks longitudinally, one of the most characteristic symptoms. Shortly after infection, conspicuous orange-pigmented stromata are produced on the outer bark and are most evident within the interior portion of the canker. The natural spread of the pathogen from infected to uninfected trees occurs primarily by conidia and ascospores, which are disseminated during the growing season by animals (e.g. insects, birds or small woodland mammals), as well as by wind or rain-splash (Anderson, 1914; Guérin *et al.*, 2001). Infection and

subsequent development within the host vary seasonally and the precise relationship between environmental conditions and the biological life-cycle of this pathogen is not fully understood (Robin & Heiniger, 2001; Guérin & Robin, 2003).

The first official record of chestnut blight disease in Spain dates back to 1947 in the Vizcaya province (Elorrieta Artaza, 1949). However, a pathogen on Japanese chestnut (*Castanea crenata* Siebold. & Zucc.) in Cógomo (Valdeorras region) that was previously (mis-)identified as *Endothiella gyrosa* Sacc. – anamorph of *E. gyrosa* [Schwein.] Fr. – could date the advent of this disease in Spain to 1940 (Molina Rodríguez, 1985) in the Galicia county (NW Spain). Also located in NW Spain, El Bierzo is an important region for nut production with more than 8000 metric tons year⁻¹, either for direct consumption or for domestic and international export, being produced. This region has been heavily infected by *C. parasitica* for many years. The first records date back to 1978 (Robredo Junco *et al.*, 1995; Viéitez Cortizo *et al.*, 1999), but the presence of dead trees and the proximity to the Valdeorras region suggest a date before this for the advent of the disease in this region. Thus, despite the advances in chestnut blight research (Bounous, 2002), it remains one of the major diseases of European chestnut in the El Bierzo region with an incidence close to 100% of the trees in many stands (Tizado *et al.*, 2010).

In terms of the economy in the region, the damage caused by chestnut blight not only affects nut and timber production, but it is also detrimental to other socio-economic sectors, such as tourism, in terms of the loss of chestnut trees from the landscape. Thus, chestnut tree mortality due to chestnut blight and the abandonment of rural areas have contributed to a reduced area of chestnut tree stands over the second-half of the 20th century, with an annual reduction rate of approximately 1%. The third Spanish National Forest Inventory indicated 18 916 ha in the El Bierzo region had *C. sativa* as the main stand species (Ministerio de Medio Ambiente, 2003) with an estimated mean tree density of 94.5 stems ha⁻¹ (Tizado *et al.*, 2010).

The regional administrative body (Junta de Castilla y León) programs the extensive infection of chestnut stands with hypovirulent strains of *C. parasitica* to control the disease in this area and to treat the infected trees. Hypovirulent strains are the result of the infection of *C. parasitica* by a cytoplasmically transmissible, single-stranded RNA virus *Cryphonectria hypovirus-1* (*Hypoviridae*) resulting in virulence attenuation (Hillman *et al.*, 1995; Hillman & Suzuki, 2004). Hypoviruses can be transmitted from hypovirulent to virulent strains through hyphal anastomosis (fusion), thereby converting virulent strains to hypovirulent (van Alfen *et al.*, 1975; Heiniger & Rigling, 1994). Hypovirulence enables the defense systems of

the trees to restrict the fungus to the outer bark (Anagnostakis, 2001). This biological control method has been applied for years in many countries of Europe (Turchetti & Maresi, 1990; Conedera, 1993; Heiniger & Rigling, 1994; Bissegger *et al.*, 1997; Robin & Heiniger, 2001; Robin *et al.*, 2010), however, currently this form of biological control is rarely used in the El Bierzo region. Natural hypovirulent strains of *C. parasitica* are only present in around 3% of the infected chestnut trees, the dominant vegetative compatibility type corresponds to the European tester EU1 comprising about 80% of isolates (Montenegro *et al.*, 2008). According to Robin & Heiniger (2001), the distribution of chestnut blight is not an indicator of chestnut blight disease severity, and from this perspective the incidence data alone may be insufficient to correctly estimate the level of disease in a given population of chestnut trees. Also, it is known that disease severity varies according to many epidemiological factors (i.e. fungus pathology, genetic features of the chestnut trees, environmental and management factors, etc.). Thus, the estimation of disease severity, as the proportion of plant that is affected, is a key aspect for the precise knowledge of the disease status, for example in order to prioritise regional biological control and/or management strategies.

There are few methodological approaches for the evaluation of disease severity in trees, and no standard or reliable approach to assess the relative severity of chestnut blight presently exists. Juhásová *et al.* (2004) classify the chestnut trees into six categories according the degree of crown damage and the number of canker wounds and propose an index of health condition (IHC) per stand as a weighted arithmetic mean of categories. Turchetti *et al.* (2008), however, used five categories supported by a specialised criteria of types of cankers and infections, but they do not quantify a disease severity value. To that end, the objective of this study was to develop a statistical methodology that accurately and efficiently estimates the disease severity using as a case study the chestnut blight in cultivated stands of *C. sativa* across the El Bierzo region.

Materials and methods

The plots established in this study were located in El Bierzo, a region of 3180 km² located in northwestern Spain (42°56'–42°17'N and 7°04'–6°06'W; Fig. 1). Plot altitude was between 355 m and 2115 m above sea-level, and the phytoclimate ranged from Mediterranean in the south and in the centre to Eurosiberian in the north. The region is relatively isolated from other regions by the Cantabrian mountain range and is only connected at altitudes below 1000 m to the Galicia county – an important chestnut growing region severely infected with



Figure 1 El Bierzo region: location of the current area of distribution of the European chestnut trees as main stand species. Bottom right, the small map shows the location of El Bierzo region (highlighted in black) in Spain.

C. parasitica (Montenegro *et al.*, 2008) – through the Sil river valley, in the west.

Survey data

A total of 7240 living chestnuts (diameter at breast height ≥ 10 cm) across 452 plots were assessed visually for the presence of *C. parasitica* and symptoms of chestnut blight between May and November 2009. Each plot was 40–70 m in diameter (mean = 50 m) and consisted of 8–30 trees (median = 17 tree per plot). All chestnuts examined were native single-stem trees, and the majority (85.1%) was grafted to high-quality scions for nut production.

Chestnut blight incidence was estimated visually according to the plant part and vertical position along the main stem: trunk (T); primary branches in the first lower quarter of the crown (A); branches in the second-lower quarter of the crown (B); and, branches in the upper half of the tree crown (C). In the survey, signs of the pathogen (fruiting bodies) and symptoms of chestnut blight disease corresponding to any type of canker (e.g. virulent and hypovirulent cankers as defined by Turchetti *et al.*, 2008), reddened branches, wilt of leaves on branches or branch die-back were observed as evidence of the disease. To retain the objective of simplicity in the evaluation protocol and to avoid the need for very specialised technicians (i.e. low financial and time costs), we did not define types of cankers and we used an ordinal scale to assess the extent of the disease on the trunk and within A, B and C sections of the crown: L0, without any visible signs or symptoms; L1, with some visible signs or symptoms but <10% of

the tree part diseased; L2, 10–25% diseased; L3, 26–50% diseased; L4, 51–80% diseased and L5, more than 80% diseased.

In addition, seven variables that relate to cultural practices were measured to determine the linkage between disease incidence and severity data in managed chestnut stands. The quantitative measurements recorded were: DBH, diameter at breast height; TBH, tree bole height; TTH, total tree height; CPA, crown projected area; CTG, number of crown tangencies with the crown of other chestnut trees and DNT, distance to the nearest chestnut tree. All the measurements were obtained using an ultrasonic hypsometer (Haglöf, Långsele, Sweden), an outside caliper, or a diameter tape. Besides the quantitative parameters, a visual assessment was made to categorise each chestnut tree on the basis of whether it was grafted to a high-quality scion for production or not (GRF).

Data analysis

Correspondence analysis (see, for example, Digby & Kempton, 1987; Legendre & Legendre, 1998; Greenacre, 2007) was used to quantify the severity of chestnut blight disease across all the 452 plots examined in this study. This multivariate analysis is a statistical exploratory technique applied mainly for graphical and numerical analysis of tables of frequencies or counts. This could be considered as an adaptation to categorical data of principal component analysis.

In the analysis of the presence/absence data in the extent of disease categories, we were mainly interested in the relationships between the disease levels in the different parts of the tree and not in the frequencies of a particular disease level. Thus, the severity analysis applied was multiple correspondence analysis (MCA) based on the complete set of pairwise cross-tabulations of variables being analysed (Burt matrix), adjusting the results of the principal inertias with the off-diagonal tables (Greenacre, 2007). The results are discussed in the context of geometric data analysis because the graphical display facilitates the intuitive understanding of the relationships among the categories of the different variables (Le Roux & Rouanet, 2005).

The relationship between the presence/absence data of chestnut blight disease and the different variables measured on each tree was analysed using generalised linear mixed models (GLMM) with a penalised quasi-likelihood (PQL) estimation (Wolfinger & O'Connell, 1993). It is known that the PQL estimators have a bias due to approximations but they are computationally straightforward for the purpose of analysing data from the 7240 trees in this study. We note that our interest lies in the linkage between tree features and the

presence/absence of chestnut blight disease, and not in its numerical quantification. GLMM was applied with a random intercept (Agresti, 2007) defining the plot as a cluster of trees because the disease response is not independent for each tree. Chestnut blight is a contagious disease, and there is a greater probability that a tree growing in the same plot as an infected tree will become infected than a tree in an uninfected plot. Moreover, there is a specific-stand effect due to more or less favourable environmental conditions (e.g. altitude, temperature, precipitation, etc) for the spread of the disease. Thus, the model fitted was

$$Y_{ij} = \beta_0 + v_j + \beta_1 X_{ij} + \beta_2 \text{GRF}_{ij} + \varepsilon_{ij}$$

where Y_{ij} represents the presence (1) or absence (0) of chestnut blight disease, X_{ij} the matrix with columns being the statistically significant measured variables included in the model, GRF_{ij} represents a dummy vector for grafted trees (1 = yes, 0 = no), v_j the random vector distributed as Normal with mean 0 and variance σ^2 for plots and ε_{ij} the error component. The constants β_0 , β_2 and the vector β_1 are parameter estimates. The index j represents the plot ($j = 1, \dots, 452$), and i represents the tree in a plot ($i = 1, \dots, n_j$; where n_j is the total number of trees in plot j).

The best GLMM was derived using approximate F -tests for the selection of the terms in a model. All the statistical analyses were carried out using R-software (R Development Core Team, 2009) running in GNU/Linux v2.6.

Results

Chestnut blight disease was present in 90.71% of the sampled chestnut plots. The 95% confidence interval for the mean percentage of trees infected per plot was 75.27–81.75% (mean = 78.51%), of which 4.26–5.99% (mean = 5.12%) were dead trees with symptoms of chestnut blight. Given that the main objective was the evaluation of disease severity only the living trees were assessed, thus avoiding the problem of identifying chestnut blight in burnt trees due to damage from fire in some plots.

Preliminary analysis

Before analysing disease levels using multivariate analysis, we examined the prevalence of chestnut blight in each part of the tree. Table 1 shows a high proportion of main stems (81.4%) without signs of the pathogen or symptoms of chestnut blight disease; however, 82.2% expressed symptoms in the crown.

Because of this different proportion, we separately analysed data from two sets of trees according to the

Table 1 Percentage of trees ($n = 7240$) expressing the six different levels of chestnut blight disease (L0–L5) based on visual assessment in the four parts of the trees

Disease level	T	A	B	C
L0	81.4	29.0	39.4	40.6
L1	4.3	15.3	15.5	23.6
L2	2.7	11.8	16.3	14.8
L3	3.6	15.1	13.6	10.6
L4	3.4	12.7	8.2	5.6
L5	4.5	16.1	7.0	4.8
Total	100.0	100.0	100.0	100.0

T, trunk; A, primary branches; B, branches in the second lower quarter of the tree crown; C, branches in the upper half of the tree crown.

DBH. Using a χ^2 -test with continuity correction for equality of proportions: for 1950 trees with a DBH ≤ 30 cm there was no significant difference ($\chi^2_{[1]} = 0.231$; $P = 0.631$) between the 47.0% of trees showing symptoms of chestnut blight disease in the crown and 47.8% in the main stem, whereas for 5290 trees with a DBH > 30 cm there was a significant difference ($\chi^2_{[1]} = 5802.635$; $P < 0.001$), with 81.5% of trees showing symptoms of chestnut blight disease in the crown compared with only 7.8% of the trees showing signs of the pathogen or symptoms of the disease in the main stem. This difference can be explained partially by the greater thickness and roughness of the bark in the main stem of chestnut trees with a DBH > 30 cm, which hides many of the symptoms of the chestnut blight disease in the trunk.

Removing main stem data from the preliminary analysis resulted in greater homogeneity when analysed using MCA; for example, Cronbach's $\alpha_1 = 0.592$ with main stem data versus $\alpha_2 = 0.914$ without these data. Cronbach's α is a coefficient widely used in social and medical sciences for assessing internal consistency or reliability of a test score for a sample of examinees (Cronbach, 1951); the direct interpretation is the expected correlation between scores (Bland & Altman, 1997). Then, the A, B, C and T scores should all measure the extent of the disease, so they should be correlated with one another. However, α_1 is clearly lower than α_2 , which indicates a high degree of consistency when removing main stem data. For this reason, the data obtained for the main stem should not be used to assess the severity of chestnut blight disease.

Evaluating the disease severity

The data (incidence) matrix used in the analysis of disease severity was made up of the 7240 chestnut trees in the study (rows) and the 18 categorical variables (columns, i.e. the six different disease levels recorded for each of

Table 2 Burt matrix (number of trees) of all pairwise cross-tabulations of the categories of the tree crown (A, B and C) and the extent of disease (0–5)

	A.0	A.1	A.2	A.3	A.4	A.5	B.0	B.1	B.2	B.3	B.4	B.5	C.0	C.1	C.2	C.3	C.4	C.5
A.0	2101																	
A.1	0	1110																
A.2	0	0	856															
A.3	0	0	0	1091														
A.4	0	0	0	0	917													
A.5	0	0	0	0	0	1165												
B.0	2082	666	80	18	5	0	2851											
B.1	17	429	525	119	23	6	0	1119										
B.2	1	9	243	756	159	13	0	0	1181									
B.3	1	5	6	196	635	145	0	0	0	988								
B.4	0	1	1	0	93	500	0	0	0	0	595							
B.5	0	0	1	2	2	501	0	0	0	0	0	506						
C.0	2022	656	165	66	25	5	2677	231	26	4	1	0	2939					
C.1	74	435	523	470	169	37	169	846	595	93	3	2	0	1708				
C.2	3	18	161	447	324	121	4	40	552	449	29	0	0	0	1074			
C.3	1	1	5	104	347	310	0	2	8	437	315	6	0	0	0	768		
C.4	1	0	2	3	51	349	1	0	0	5	246	154	0	0	0	0	406	
C.5	0	0	0	1	1	343	0	0	0	0	1	344	0	0	0	0	0	345

the three tree crown parts) being analysed after removing the main stem data. Therefore, the evaluation of disease severity is derived from the tree crowns. The Burt matrix, derived from the incidence matrix, made up of the cross-classification counts of trees per category, is given in Table 2.

Fig. 2 shows the geometric representation of the columns and rows of the incidence matrix (categories and individual trees) on the first two axes from the MCA. Using the criteria of mean eigenvalue $\lambda_m = 0.333$ (the average inertia per dimension, λ_m , serves as a threshold for deciding which axes are worth interpreting in MCA), we could select these two axes ($\lambda_1 = 0.853$ and $\lambda_2 = 0.612$), which together explain 74.3% of the total inertia. The observed horseshoe effect in the two-dimensional representation indicates that near neighbours, that is trees, are related as regards the disease classification as expected in a contagious disease. In addition, the horseshoe effect together with the one-dimensional model of the classification criteria used to measure the disease extension clearly reflects a unique latent variable to explain these data. According to Warrens & Heiser (2006), the first solution of a MCA of a monotonic model contains relevant information about the items, that is, trees (rows in the data matrix), and the use of two-dimensional plots should be unnecessary. Hence, Table 3 only shows the contributions and standard coordinates for the first axis.

The total contribution for each of the A, B and C parts of the tree crown (columns) is similar to the expected 1/3 and therefore these three distinct parts are not relevant to characterise the disease severity. However,

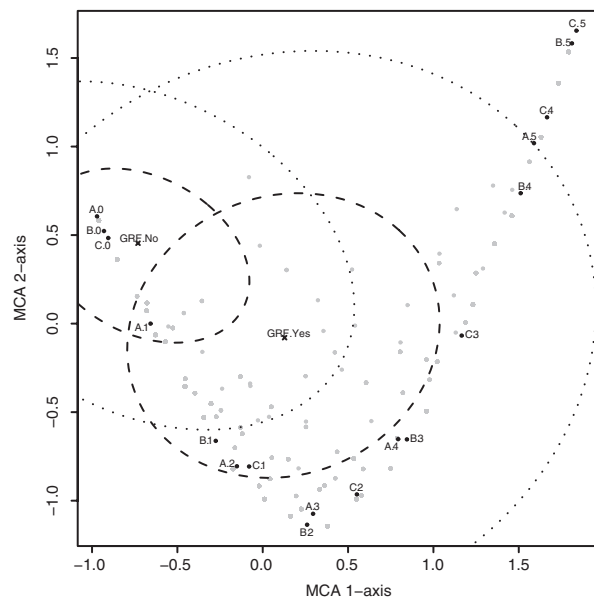


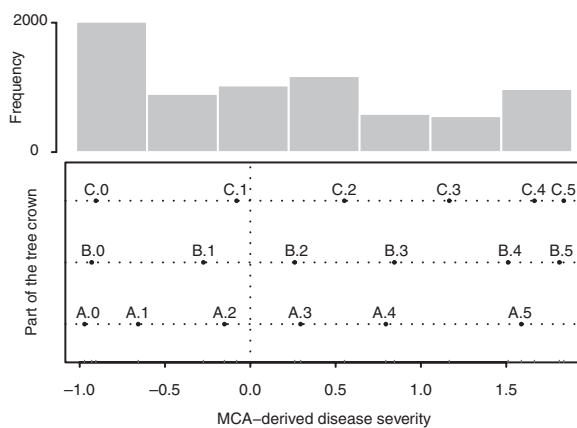
Figure 2 Multiple correspondence analysis (MCA) of the categories of disease severity (black dots) and individual trees (grey circles) in the first two axes. Information from the supplementary variable GRF with two levels (Yes or No) is also shown as a bivariate Normal distribution given the two MCA axes, with ellipses having interval of half-length $\sigma_{MCA1} = 0.915$ and $\sigma_{MCA2} = 0.804$ for GRF.Yes ($\rho = 0.096$) and $\sigma_{MCA1} = 0.615$ and $\sigma_{MCA2} = 0.492$ for GRF.No ($\rho = -0.296$) (dashed ellipses), and also with ellipses of half-length twice these σ_{MCA1} and σ_{MCA2} values (dotted ellipses).

there is a strong opposition between the coordinates of the crown parts showing no symptoms (L0) and those showing disease symptoms in more than 80% of the

Table 3 Basic results of the multiple correspondence analysis (MCA) for the first axis (eigenvalue = 0.853). For the three parts of the tree crown (A, B and C) and the six extent of disease categories (L0–L5) is shown

Disease level	A			B			C			Total inr
	inr	coor	qlt	inr	coor	qlt	inr	coor	qlt	
L0	0.068	-1.05	0.63	0.079	-1.01	0.64	0.073	-0.98	0.66	0.220
L1	0.018	-0.71	0.41	0.045	-0.30	0.20	0.044	-0.09	0.40	0.107
L2	0.029	-0.16	0.31	0.052	0.28	0.48	0.038	0.60	0.54	0.119
L3	0.042	0.32	0.51	0.057	0.91	0.31	0.046	1.26	0.35	0.145
L4	0.045	0.86	0.33	0.058	1.64	0.46	0.044	1.80	0.60	0.147
L5	0.097	1.72	0.66	0.091	1.96	0.50	0.074	1.99	0.44	0.262
Total inr	0.299			0.382			0.319			1.000

inr, relative contribution to total inertia of the axis; coor, standard coordinates; qlt, quality of representation in the two-dimensional space (0–1).

**Figure 3** Tree histogram and principal coordinates for the first axis of the multiple correspondence analysis (MCA) of each part of the tree crown (A, B and C) and extent of disease categories (0–5).

crown part (L5), with both of these categories making a significant contribution to the axis within each of A, B and C, greater than the expected 1/6. Moreover, the one-dimensional representation of the individuals on this axis (Fig. 3) shows the principal coordinates for each part of the tree crown (A, B and C) on a continuous gradient from L0 (negative values) to L5 (positive values). The interpretation of the first axis in terms of MCA is that it mirrors the severity of the disease, with severe damage on the right side (crown with categories of L5) and healthy on the left side (crown with categories of L0). Hence, the first MCA dimension can explain at least the intensity of disease in the tree crown (43.2% of the total inertia) and the coordinates along the axis seem to be a reasonable approximation of the underlying latent variable: severity of disease.

The standard coordinates (Table 3) can be used to quantify the disease severity score of a tree. The value can be calculated as the arithmetic mean of the standard coordinates for the extension level in each part of the tree.

For example, a tree with A/L2 (-0.16), B/L1 (-0.30) and C/L0 (-0.98) gives a disease severity score of -0.48. As these values range from -1.01 (trees with L0 in all parts of the crown) to 1.89 (tree with L5 in all parts of the crown), we can rescale to the range 0 to 1 giving the value of 0.18 for disease severity in this tree.

Disease and tree features

The linkage between the presence/absence of chestnut blight disease and tree metrics was analysed using the individual data of the 7240 trees and the seven variables (six quantitative + one qualitative).

Of particular interest is the qualitative factor GRF because of the general idea that cultural techniques may be important for the incidence and dissemination of the disease (Bragança *et al.*, 2009). Table 4 shows means and standard errors for the quantitative variables grouped by this factor. All the variables except TTH show significant differences ($P < 0.001$) between grafted and non-grafted trees using two-sample *t*-test. These variables show that grafted trees have reduced tree bole height (TBH), greater trunk diameter (DBH), a wider tree crown (CPA), and are more widely spaced in the plot (DNT), compared to non-grafted trees, which is to be expected for trees growing in the managed nut production stands of this region.

In the analysis of presence of chestnut blight disease by GLMM, the tree height (TTH and TBH) and the crown tangencies (CTG) are variables that do not show statistical significance ($P > 0.05$, *F*-tests). After removing these variables, the resulting fitted model includes GRF, DBH, DNT and CPA variables. Table 5 shows the GLMM results for the final model. The estimated intraclass correlation coefficient (ICC = 0.97) shows, as expected, a specific-stand effect for this contagious disease. The GLMM results indicate that grafted trees have 3.92 times higher probability of infection than non-grafted ones. A simple analysis of the 2 by 2 table of the GRF variable

Table 4 Descriptive statistics (means and standard errors) for measured chestnut tree features for the 7240 trees grouped by the whether the trees were grafted or not (GRF). Significant differences between GRF.No and GRF.Yes using two-sample *t*-test (d.f. = 7238)

GRF	No (<i>n</i> = 1079)		Yes (<i>n</i> = 6161)		<i>t</i> -Value	<i>P</i>
	Mean	SE	Mean	SE		
DBH	20.7	0.63	60.0	0.47	34.47	<0.001
TBH	3.1	0.07	2.3	0.01	19.14	<0.001
TTH	12.0	0.18	11.9	0.06	0.65	0.590
CPA	41.0	1.13	92.6	0.73	28.73	<0.001
CTG	2.5	0.05	2.1	0.02	7.29	<0.001
DNT	5.0	0.09	8.0	0.04	31.15	<0.001

DBH, diameter at breast height (cm); TBH, tree bole height (m); TTH, total tree height (m); CPA, crown projected area (m²); CTG, number of crown tangencies with the crown of other chestnut trees; DNT, distance to the nearest chestnut tree (m).

Table 5 Generalised linear mixed model (GLMM) for the presence/absence of chestnut blight by tree metrics (7240 trees grouped in 452 plots; d.f. = 6783).

	Value	SE	<i>t</i> -Value	<i>P</i>
DBH	0.0238	0.002	10.68	<0.001
CPA	0.0105	0.001	8.24	<0.001
DNT	0.0386	0.014	2.62	0.009
GRF	1.3670	0.238	5.75	<0.001
DBH.CPA	-0.0001	0.000	9.19	<0.001

DBH, diameter at breast height; CPA, crown projected area; DNT, distance to the nearest chestnut tree.

according to the presence or absence of the disease shows that the mean probability of a non-grafted tree being infected is 0.44 versus 0.82 for grafted trees, that is, these values indicate that the relative risk of acquiring the disease is 87.9% higher for grafted trees.

Fig. 2 shows GRF as a supplementary variable in MCA. As expected by the relative risk of the disease, there is a clear association between non-grafted trees (GRF.No) and low disease severity values, whereas the grafted trees (GRF.Yes) are associated with medium disease severity values. However, note that grafted trees show a wider range of variation from low levels of MCA-derived disease severity to severely affected, reflecting more variable extensions of the disease on these trees. The results of GLMM agree with the statistical method applied to evaluate disease severity (MCA) showing GRF as an important factor related to the presence of the blight disease. Also, Fig. 4 shows a nonlinear relationship between prevalence (proportion of infected trees) and the mean of the MCA-derived disease severity of the trees in the plots, that is, both variables do not reflect the same kind of information.

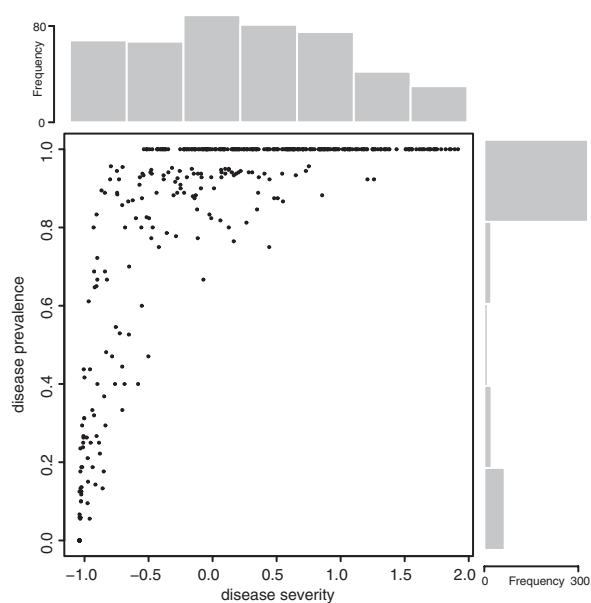


Figure 4 Scatterplot and histograms of the prevalence and the mean of the disease severity (coordinates of the first axis of the MCA) for the 452 plots studied.

Discussion and conclusions

Chestnut blight disease was present in 90.7% of plots examined in El Bierzo and the estimated percentage of trees infected is 78.5%. These data show that a large proportion of the chestnuts in the study area were diseased by *C. parasitica* and that chestnut blight disease is widespread. The prevalence is similar to that found in locations in other countries, for example, 67–99% in Italy (Amorini *et al.*, 2001; Turchetti *et al.*, 2008) or around 80% in Iran (Ghezi *et al.*, 2010), though higher than in others such as Portugal with 17–65% infected trees (Bragança *et al.*, 2009). The results of this investigation through GLMM showed that diameter at breast height, crown projected area, distance to the nearest tree, and whether the tree is grafted or not were important in the explanation of disease incidence; all of them related to cultural practices in managed stands.

The prevalence data as shown by the high estimated proportion of trees infected inside plots contrasts with the data on the severity of the disease. Fig. 4 shows that the contagious nature of this disease results in a prevalence histogram with high values in both tails, whereas the severity histogram shows a distribution more consistent with the progressive expansion of a contagious disease in an area. Thus, the mean MCA-derived severity value inside a plot (rescaled to the range 0–1) was 0.371 and the third quartile was equal to 0.621. Furthermore, 15.48% of the plots have a severe damage

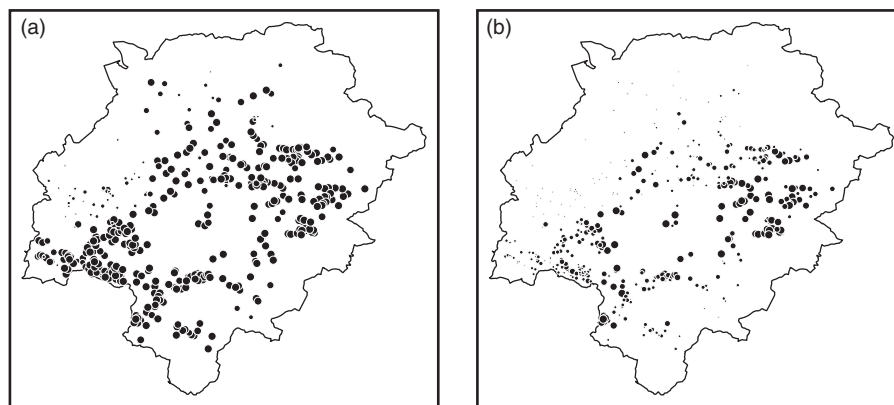


Figure 5 A comparison of the prevalence and severity of the chestnut blight disease in El Bierzo: (a) prevalence calculated as the percentage of the trees in the plot showing signs of the pathogen and/or symptoms of the disease and (b) plot-level severity calculated as the mean of the MCA-derived disease severity. Each black spot indicates the location of one of the 452 sampled plots.

higher than 0.75 severity, and the severity histogram in Fig. 3 shows that 40.2% of the trees are in the first (left) two bars. Hence, using presence or absence data alone to assess the importance of the disease in an area can be misleading. This difference is even more significant when it is illustrated on disease maps. The prevalence map (Fig. 5a) shows a similar incidence of the chestnut blight disease in almost the entire region except a small area in the west; whereas, the severity map (Fig. 5b) shows a heterogeneous distribution with many severely damaged trees in the northeast. From the perspective of the disease management, the distribution of prevalence data presents a different scenario than the distribution of severity data. Thus, a simple prevalence map of chestnut blight disease distribution is not an efficient indicator of chestnut blight importance in an area unless it takes into account other evaluation parameters of the health condition of the stands (e.g. presence of hypovirus or mortality). Disease severity analysis provided a more accurate estimation of the relative state of the disease and permitted a more accurate assessment of the importance of the infection in an area without specialised sample protocols.

The disease severity analysis, using categories on an ordinal scale, relates to other important biological information such as tree (i.e. GRF) or environmental (i.e. location) variables, or the generic spread of the disease within an infected tree. For example, Fig. 3 shows that the disease severity increases (L0 to L5) as the disease spreads from A, to B to C in the crown. Note that the levels of disease in A part of the tree are close to the previous B or C levels, and B levels to the previous same C level. This spreading of disease within the canopy suggests that the penetration of the pathogen is located in the lower part of the crown (A) and spreads to the tips of the branches in the upper part of the crown (C).

This expansion is the opposite of what generally happens when a tree is infected by other systemic pathogens such as some bacteria or fungi, which mainly use small injuries as penetration channels and spread from the tips to the primary branches and the main stem (e.g. fire blight). Given that the most common cause of injuries in chestnuts is the deliberate grafting and pruning of primary or basal branches as part of the management of trees for nut production, our results suggest that man-made wounds are the main channel of pathogen penetration. There are other natural or artificial causes of infection due to the breaking of branches by climatological events, or minor injuries at the axil between different order branches (or adventitious shoots) where parenchyma is easily subjected to wounding by free oscillation, but deliberate wounding clearly enhances infection.

The time variable is not included in the methodology to evaluate the disease severity, that is, it is not possible to use the severity data from only one time point to forecast the evolution of the disease in a plot (the same occurs with prevalence data because both change over time). For example, suppose a simple scenario of two geographically separated plots, each with an equal number of living stems of chestnut and high disease incidence. The number of cankers per tree and their extension in the crown are also roughly equal and significant branch die-back exists throughout each plot. However, if one plot had hypovirus-containing cankers (natural spread or biological control) while all the cankers in the other plot possessed virulent cankers, according to the current protocol, the disease severity index for both plots would not be substantially different and would likely indicate that these plots are severely affected by chestnut blight. Only a sample protocol with a specialised criteria to identify types of canker permits the information

on hypovirulence to be obtained; so the better life expectancy of the trees in the plot with hypovirus are not revealed by the present approach. However, to confirm the control of the disease by the hypovirulent strains, it would be necessary to take samples for analysis to determine canker status (another specialised sample) resulting in two expensive studies in terms of time and money. From the perspective of the management, similar knowledge could be obtained with the current approach and two samples over time, for example, analysing the variation over time in the (tree or plot) severity index between samples (mean comparisons, statistical distribution analysis, . . .) or using the data of the second sample as supplementary rows (trees) in the MCA of the first sample. Moreover, the cost of this methodology of evaluation is significantly lower than a specialised study because this is simple enough, more rapid and avoids the need for very specialised technicians in the field.

This methodology should be objective and efficient to obtain accurate information for a wide and repeated application. Continued monitoring of the chestnut blight disease and its causal agent *C. parasitica* is critical in order to collect biologically important information about the pathogen (e.g. the rate of spread of the pathogen or the impact of hypovirulent strains) and the effectiveness of the disease remediation strategies. The base of the crown should be targeted to evaluate the health of the tree, at least while cultural practices remain the key factor in the disease penetration. Moreover, methodologies that do not require field checking of individual trees (e.g. remote sensing) still do not accurately indicate the importance of disease because the symptoms in the upper part of the crown appear in the later stages of the disease.

The analysis of disease severity using MCA with simple categories allows a disease index to be constructed. A modelling approach (GLMM) allows the variables relevant to the incidence of the disease to be discovered and explored. The methodology is simple enough to apply and could be appropriate for other plant (or animal) diseases with external and recognizable signs and/or symptoms. Nevertheless, the disease severity analysis should initially consider the specific factors of the plant (or animal) disease and the linkage between the categorical variable (e.g. extent of disease) and the severity of the disease.

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References

- Agresti A. (2007) *An Introduction to Categorical Data Analysis*. 2nd edn. Hoboken, NJ: John Wiley & Sons, Inc.
- Anagnostakis S.L. (2001) American chestnut sprout survival with biological control of the chestnut-blight fungus population. *Forest Ecology and Management*, **152**, 225–233.
- Anderson P.J. (1914) The morphology and life history of the chestnut blight fungus. *The Commission for the Investigation and Control of the Chestnut Tree Blight Disease in Pennsylvania*, Bulletin no. 7. Harrisburg, PA: W.M. Stanley Ray, State Printer.
- Amorini E., Manetti M., Turchetti T., Sansotta A., Villani F. (2001) Impact of silvicultural system on *Cryphonectria parasitica* incidence and on genetic variability in a chestnut coppice in Central Italy. *Forest Ecology and Management*, **142**, 19–31.
- 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.
- Bland J.M., Altman D.G. (1997) Cronbach's alpha. *British Medical Journal*, **314**, 572.
- Bounous G. (2002) *Inventory of Chestnut Research, Germplasm and References*. Rome, Italy: FAO and Agriculture Organization of the United Nations.
- Bragança H., Simões S., Onofre N., Santos N. (2009) Factors influencing the incidence and spread of chestnut blight in northeastern Portugal. *Journal of Plant Pathology*, **91**, 53–59.
- Conedera M. (1993) *Cancro corticale del castagno*. Berichte der Eidgenössischen Forschungsanstalt für Wald, Schnee und Landschaft, Birmensdorf, Switzerland. p. 335.
- Cronbach L.J. (1951) Coefficient alpha and the internal structure of tests. *Psychometrika*, **16**, 297–334.
- Digby P.G.N., Kempton R.A. (1987) *Multivariate Analysis of Ecological Communities*, London, UK: Chapman & Hall.
- Elorrieta Artaza J. (1949) *El Castaño en España*. Madrid, M.A.P.A., España: Instituto Forestal de Investigaciones y Experiencias.
- Ghezi E., Khodaparast A., Zare R. (2010) Distribution and severity of damage by *Cryphonectria parasitica* in the chestnut stands in Guilan province, Iran. *Forest Pathology*, **40**, 450–457.
- Greenacre M. (2007) *Correspondence Analysis in Practice*. 2nd edn. Boca Raton, FL: Chapman & Hall; CRC.
- Griffin G.J. (1986) Chestnut blight and its control. *Horticultural Reviews*, **8**, 291–335.
- Gryzenhout M., Wingfield B.D., Wingfield M.J. (2006) New taxonomic concepts for the important forest pathogen *Cryphonectria parasitica* and related fungi. *FEMS Microbiology Letters*, **258**, 161–172.
- Guérin L., Froidefond G., Xu X.M. (2001) Seasonal patterns of dispersal of ascospores of *Cryphonectria parasitica* (chestnut blight). *Plant Pathology*, **50**, 717–724.

- Guérin L., Robin C. (2003) Seasonal effect on infection and development of lesions caused by *Cryphonectria parasitica* in *Castanea sativa*. *Forest Pathology*, **33**, 223–235.
- Hebard F.V., Griffin G.J., Elkins J.R. (1984) Developmental histopathology of cankers incited by hypovirulent and virulent isolates of *Endothia parasitica* on susceptible and resistant chestnut trees. *Phytopathology*, **74**, 140–149.
- Heiniger U., Rigling D. (1994) Biological control of chestnut blight in Europe. *Annual Review of Phytopathology*, **32**, 581–599.
- Hepting G.H. (1974) Death of the American chestnut. *Journal of Forest History*, **18**, 60–67.
- Hillman B.I., Fulbright D.W., Nuss D.L., Van Alfen N.K. (1995) Hypoviridae. In *Sixth Report of the International Committee for the Taxonomy of Viruses*. Eds F.A. Murphy, C.M. Fauquet, D.H.L. Bishop, S.A. Ghabrial, A.W. Jarvis, G.P. Martelli, M.A. Mayo and M.D. Summers. New York, NY: Springer-Verlag.
- Hillman B.I., Suzuki N. (2004) Viruses of the chestnut blight fungus, *Cryphonectria parasitica*. *Advances in Virus Research*, **63**, 423–472.
- Juhássová G., Adamcikova K., Ivanova H., Kobza M. (2004) Situation of damage caused by *Cryphonectria parasitica* to forest stands and orchards of *Castanea sativa* by 2001 in Slovakia. *Horticultural Science*, **31**, 102–108.
- Legendre P., Legendre L. (1998) *Numerical Ecology*. Amsterdam, The Netherlands: Elsevier.
- Le Roux B., Rouanet H. (2005) *Geometric Data Analysis. From Correspondence Analysis to Structured Data Analysis*. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Ministerio de Medio Ambiente (2003) *Mapa Forestal de España a Escala 1:50.000 de la Provincia de León*. Madrid, España: Organismo Autónomo Parques Nacionales.
- Molina Rodríguez F. (1985) Cuarenta años de investigación sobre el castaño en el departamento forestal de Lourizán. In *Congreso Internacional sobre el Castaño*. Ed. Lourizán. Consellería de Agricultura, Pesca y Alimentación. Santiago de Compostela, España: Xunta de Galicia.
- Montenegro D., Aguín O., Sainz M., Hermida M., Mansilla J. (2008) Diversity of vegetative compatibility types, distribution of mating types and occurrence of hypovirulence of *Cryphonectria parasitica* in chestnut stands in NW Spain. *Forest Ecology and Management*, **256**, 973–980.
- R Development Core Team (2009) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Robin C., Heiniger U. (2001) Chestnut blight in Europe: diversity of *Cryphonectria parasitica*, hypovirulence and biocontrol. *Forest Snow and Landscape Research*, **76**, 361–367.
- Robin C., Lanz S., Soutrenon A., Rigling D. (2010) Dominance of natural over released biological control agents of the chestnut blight fungus *Cryphonectria parasitica* in south-eastern France is associated with fitness-related traits. *Biological Control*, **53**, 55–61.
- Robredo Junco F., Berrocal del Río M., Cardeñoso Herrero J.M. (1995) El chancro del castaño (*Cryphonectria parasitica*) en Castilla y León. *Montes*, **42**, 32–42.
- Tizado E.J., Terrón Alfonso A., Núñez-Pérez E. (2010) *Valoración del estado fitosanitario actual del castaño europeo en El Bierzo (León, España)*. Ponferrada, España: Fundación Ciudad de la Energía.
- Turchetti T., Ferretti F., Maresi G. (2008) Natural spread of *Cryphonectria parasitica* and persistence of hypovirulence in three Italian coppiced chestnut stands. *Forest Pathology*, **38**, 227–243.
- Turchetti T., Maresi G. (1990) Indagini sulla diffusione naturale degli isolati ipovirulenti di *Cryphonectria parasitica* in alcuni cedui di castagno. *Atti delle Giornate Fitopatologiche*, **2**, 89–98.
- Van Alfen N.K., Jaynes R.A., Anagnostakis S.L., Day P.R. (1975) Chestnut blight: biological control by transmissible hypovirulence in *Endothia parasitica*. *Science*, **189**, 890–891.
- Viéitez Cortizo E., Viéitez Madriñán M.L., Viéitez Madriñán F.J. (1999) *O castiñeiro: bioloxía e patoloxía*. Santiago de Compostela, España: Consello da Cultura Galega.
- Warrens M.J., Heiser W.J. (2006) Scaling unidimensional models with multiple correspondence analysis. In *Multiple Correspondence Analysis and Related Methods*. Eds M. Greenacre and J. Blasius. New York: Chapman & Hall; CRC.
- Wolfinger R., O'Connell M. (1993) Generalized linear mixed models: a pseudo-likelihood approach. *Journal of Statistical Computation and Simulation*, **4**, 233–243.