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FACTORES AMBIENTALES EN LA DISTRIBUCIÓN DE LA SECA EN LA ENCINA (*QUERCUS ILEX SUBSP. BALLOTA*)

ENVIRONMENTAL VARIABLES IN THE DISTRIBUTION OF *LA SECA* DISEASE IN THE HOLM OAK (*QUERCUS ILEX SUBSP. BALLOTA*)

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Resumen

La combinación de factores bióticos y abióticos (clima, orografía, litología, competencia interespecífica, etc.) y la patogenicidad de agentes como el hongo *Phytophthora cinnamomi* Rands han generado la enfermedad fitosanitaria denominada en España como *la seca* de encinas y alcornoques de la Península Ibérica. El objetivo del presente estudio es el análisis que desempeñan las variables ambientales en el desarrollo de dicha enfermedad y determinar cuáles tienen una mayor influencia en su propagación. Se toma como área de estudio el Monte de Valdelatas, Alcobendas, Madrid (hoja 534 del Mapa Topográfico Nacional a escala 1:50.000. Coordenadas UTM X: X: 442592.51, Y: 4487266.63 zona 30T). Se obtivieron muestras en campo de 100 árboles de *Quercus ilex* subsp. *ballota* (50 con apariencia saludable y 50 con síntomas aparentes de enfermedad) azarosamente ubicados en 7 transectos. En relación al modelo estadístico se empleó un modelo binomial cuya variable respuesta se definió como sano/enfermo, según la apariencia del árbol. Así mismo, se manejó el Criterio de Información de Akaike (AIC) con el fin de establecer el mejor modelo. En su caso, presentando un 44% de la varianza (D^2 = 0.437) y un valor de 89.68 en AIC. Las principales variables ambientales que interfieren en la enfermedad fueron: proximidad a cursos fluviales, índice de humedad topográfico, cobertura de *Daphne gnidium* y *Quercus ilex* subsp. *ballota* y la edad de los individuos muestreados. Dada la pérdida en la masa forestal de *Quercus* debido a la enfermedad de *la seca*, es necesario comprender el comportamiento de dicha enfermedad, así como conocer los factores ambientales que contribuyen a su expansión, pudiendo llegar de tal modo a identificar algún factor inhibidor.

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Palabras clave

Formaciones forestales de *Quercus*; variables ambientales; *Daphne gnidium;* índice topográfico de humedad; decaimiento de la encina; interior de España.

Abstract

The combination of biotic and abiotic factors (climate, orography, lithology, interspecific competition, etc.) and the pathogenicity of agents such as the fungus *Phytophthora cinnamomi* Rands has generated the phytosanitary disease known as the oak decline (*la seca*) in holm oak and cork oak stand of the Iberian Peninsula. The aim of this study is to analyse the role played by environmental variables in the development of this disease, and to determine which have a stronger influence in the spread of the disease. Data has been obtained from Monte de Valdelatas, Alcobendas, Madrid (sheet 534 national topographic map, scale 1:50.000, UTM coordinates X: 442592.51, Y: 4487266.63 zone 30T). Field samples were taken from 100 randomly selected *Quercus ilex* subsp. *ballota* individuals (50 with a healthy appearance and 50 that showed disease symptoms), located within 7 transects. In relation to the statistical model, in this work a binomial model was used. On it, the response variable was defined as healthy/unhealthy, depending on the appearance of each tree. Akaike Information Criteria (AIC) was used to define the quality of the model. We selected model obtained a score of 89.68 and accounts for approximately 44% of data variability ($D^2 = 0{,}437$). The main environmental variables that impact disease were: proximity to river course, topographic wetness index, cover of *Daphne gnidium* and *Quercus ilex* subsp. *ballota*, and the age of the individuals. Given the loss of *Quercus* forest mass caused by the spread of the oak decline disease, detected in several countries, including Spain, it becomes necessary to adequately understand the different factors that contribute and, in some cases, is the cause of the expansion of the disease, as well as to identify any inhibiting factors.

Keywords

Quercus forests; environmental variables; *Daphne gnidium*; topographic wetness index; *oak* decline; central Spain.

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INTRODUCTION

Sclerophyllous forests of holm oak and cork oak, the dominant forests in the Iberian Peninsula, are experimenting a significant regression since the 1980's (Herranz, 2004; Cobos *et al*., 1993; Brasier, 1996; Arias and Del Pozo, 1997; Moreira *et al*., 2006).that is caused by the affection, decay and death of the woodland due to the Phytosanitary condition called *la seca* (Tuset and Sánchez, 2004; Cobos *et al*., 1993).

These plant communities show a great functional singularity due to their adaptation to multiple climatic stresses: winter temperatures, irregular rainfall, and a summer period that coincides with maximum drought and higher thermal rigor. From an edaphic point of view, the scarcity of nutrients in the soil contributes to the development of adaptations (Blanco *et al.,* 1997), such as the presence of a thick cuticle to avoid moisture loss during the dry season, a large foliar surface (allowing for greater light absorption), a significant reduction in metabolic activity below 10ºC, storage of nutrients in the wood for long periods of time, and a penetrating root system that facilitates usage of underground water at 6-8m depth (Terradas *et al.*, 1980; Escarré *et al.*, 1984). The trend towards a climate scenario with long periods of drought, rise of temperature, and a more sudden transition between humid and dry climatic phases, are factors that worsen the general state of plant cover (Allué, 1995; Manrique and Fernández, 2000; Fernández *et al*., 2001) and facilitate the proliferation of pathogens that cause diseases such as *la seca*.

This phytosanitary disease involves pathogens such as the fungus *Phytophthora cinnamomi* Rands that is active on the tree roots and hinders water absorption (Brasier *et al.,* 1993; Cobos *et al.,* 1993; Tuset *et al.*, 1996). This pathogen is considered responsible for the majority of infestation foci in the Iberian Peninsula (Tuset *et al.*, 1996; Rodriguez-Molina *et al.*, 2002 and 2003).

Phytophthora cinnamomi Rands needs live plant tissue as food source, and free water to form its reproductive organs. It therefore infects plant tissue where there is moisture present (Brasier *et al*., 1993). The most favourable locations for the fungus development are valleys or depressions in poorly drained terrain associated to marl, silt or clay soils (Duniway,1983), with a pH approximately 5 and a topsoil rich in organic matter (Jung *et al*., 2000), that show moisture values between 5% and 19% except for periods of summer drought (Tuset *et al*., 2002), and temperatures that range from 5ºC to 35ºC, as well as the presence of different plant species needed for the distribution and survival of the pathogen.

Several factors contribute to the development of the disease. Executive factors, that constitute pathogenic organisms that act causing the death of the affected individual. Catalyst factors or environmental variables such as the presence of moisture for prolonged time periods, soils with high of organic matter content, or topographic depressions that favor the dispersion of *la seca* (Montoya and Mesón, 1994). Finally, predisposing factors, such as the interspecific competition and the age of the individual, whereby older individuals are at higher risk of suffering *la seca*.

One of the most interesting biotic variables in this study is the shrub and tree strata. The presence of affected species eases the transmission to other nearby organisms with no apparent symptoms, subject to the dispersion capability of the pathogen (Montoya, 1981). The presence of certain species in the shrub stratum favors or inhibits the proliferation of *Phytophthora cinnamomi* Rands. Thus, *Cistus crispus* or *Cistus ladanifer* are considered favoring species, the latter being considered a possible *Phytophthora cinnamomi* reservoir (Sampaio *et al*., 2013). On the other hand, other species such as *Daphne gnidium*, *Helychrisum stoechas* and *Lavandula stoechas* possess antimicrobial defenses and thus reduce or avoid altogether pathogen growth, and therefore hinder the propagation of *la seca*. Such species may be used as bioindicators (Cardillo and Acedo, 2013).

The combination of predisposing and catalyst factors, together with the presence of *Phytophthora cinnamomi*, generate a characteristic set of physical traits in the trees and the subsequent death of the individuals, as the disappearance of foliage, the partial or total appearance of dry branches and, on occasions, liquen colonization. It is so-called progressive decay (Tuset *et al*., 2006) (Figures 1 and 2).

FIGURES 1 AND 2. INDIVIDUALS AFFECTED BY *LA SECA* IN MONTE VALDELATAS; PROGRESSIVE DECAY CAN BE SEEN. RESPECTIVELY: INDIVIDUAL WITH PRESENCE OF LICHENS ON ITS DRIED BRANCHES. A COMPLETELY DRIED INDIVIDUAL DUE TO THE DISEASE, NO LICHEN PRESENT ON ITS BRANCHES.

This study addresses the analysis of catalyst and predisposing factors that are involved in the distribution of *la seca* in a specific location: Monte Valdelatas (Madrid). This location is in continuity to the Monte El Pardo holm oak grove, the most important Mediterranean forest in the region of Madrid.

STUDY AREA

The area under study is within the municipal limits of Alcobendas (Madrid, Spain, sheet 537 National Topographic Map, Scale 1:50000, coordinates UTM en X: 442592.51 e Y: 4487266.63 zone 30T) and the city of Madrid (see Figure 3). From a geological perspective, it is located in the tertiary basin of Madrid, bound by reverse faults of great development. It constitutes a narrow channel dug into the ground (Graben), due to reactivation of the fragile structures from the late Hercynian period, and with an active Cenozoic sedimentation border that corresponds to the Sierra de Guadarrama margins (Llamas and López, 1975). The area presents a soft topography, with small elevations and depressions. It has experimented a progressive filling since the Miocene, with the sediments from the mountain ranges that surround it (Bascones *et al*., 1986). Lithologically it is characterised by the generalised presence of coarse Arkose and blocks of granite on Monte El Pardo (Bascones *et al.*, 1986). It is located in the Tajo depression (Pérez, 1994) with altitude in the range of 600-700 m. The climate is continental Mediterranean (mean temperature of 13.7ºC and annual rainfall of 444 millimeters) characterized by a high thermal range between seasons and large interannual rainfall fluctuations. The hydrographic network is made up of small streams that drain to the Jarama basin, some constituting drainage residual waters from several buildings located in Monte Valdelatas. This drainage could increase the organic water composition, who we express in the results, a high number of trees focus near this course. Human activities have transformed the natural habitat of this dehesa, composed mainly of *Quercus ilex* subsp. *ballota* (Génova, 1989). In 1928, the poor state of the Monte Valdelatas and especially of the arborea strata, causes a reforestation process. Nevertheless, in the Guerra Civil (1936-1939) it was razed. From 1942, the government initiated reforestation processes whit *Pinus pinea, Pinus pinaster* and *Quercus ilex* subsp. *ballota* trees (Venturas *et al.,* 20011). So, nowadays the vegetation is composed of holm oak trees and reforested pines, with frequent areas of mixed species areas, and riparian communities. In many places these arboreal communities have been replaced by shrub species such as *Cistus ladanifer*, *Cytisus scoparius* and ruderal nitrophilous ones, or else by of ruderal herbaceous communities, nitrophilous species, weeds (Génova, 1989).

MATERIAL AND METHODS

After detailed field reconnaissance, we proceeded to the detection, identification, georeferencing and mapping of *Quercus ilex* subsp*. ballota* individuals affected by *la seca* disease. In order to arrive at conclusive results, environmental variables data from affected trees had to be compared to that from healthy trees. 100 individuals were randomly selected, 50 showing symptoms of progressive decay and 50 with no symptoms. In both cases, variables potentially related with *Phytophthora cinnamomi* affection were accounted.

Detection of individuals under study involved plotting 7 transects of 20 meters width and an approximate accumulated length of 7 kilometers (Figure 3). These covered a large area of the holm oak stand and areas of mixed holm oak and reforested pines, in areas nearby and distant to fluvial courses. For each individual within transect, geographic coordinates were recorded with GPS, and a cartographic representation using GIS (ArcGIS) was obtained. (Figure 3).

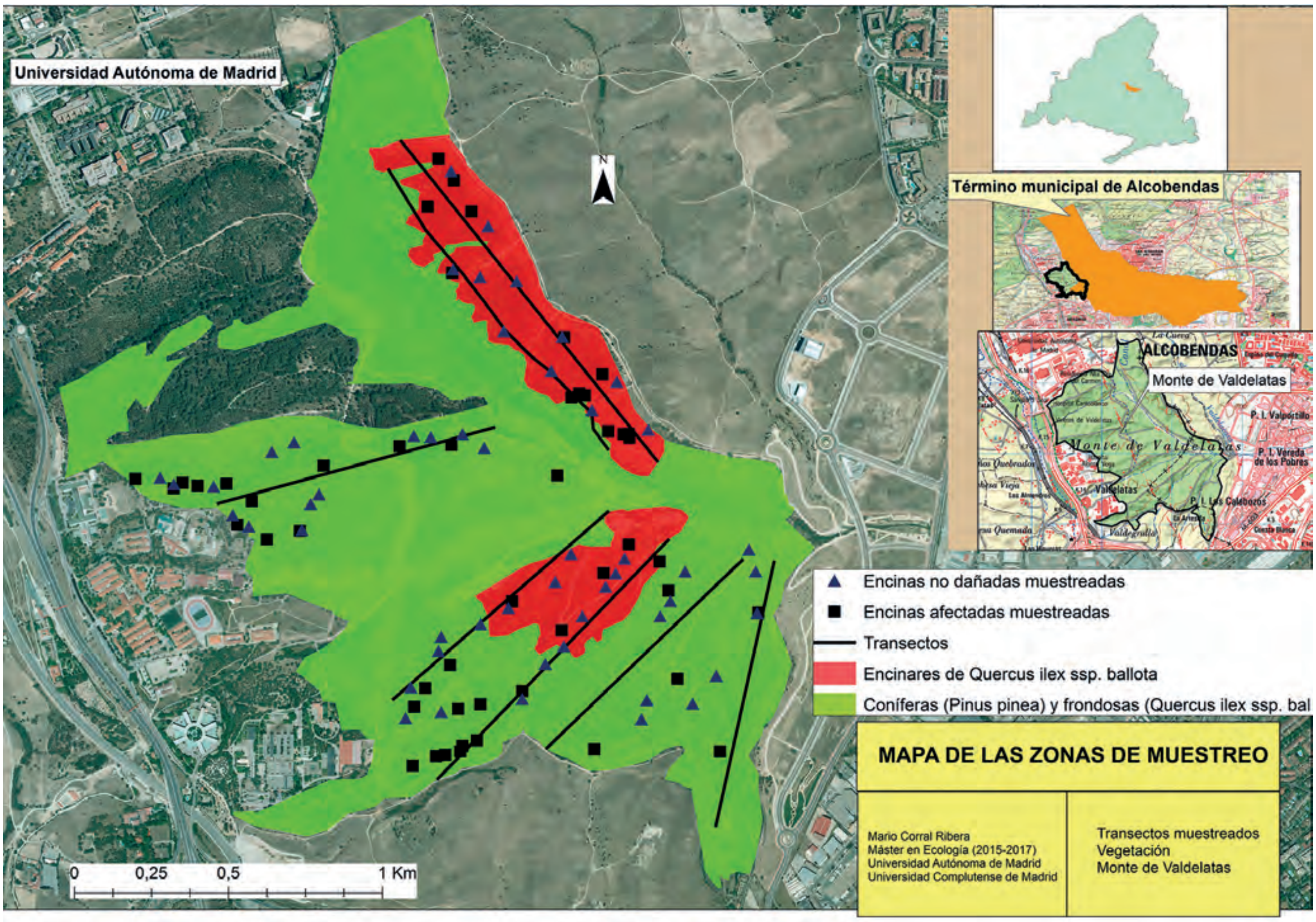

FIGURE 3. MAP OF THE AREA UNDER STUDY.

On the other hand, and considering that *la seca* disease shows great dispersion capability amongst holm oak individuals (Montoya and Mesón, 1993), the distance to the nearest affected individual was measured for each selected tree. We considered the following as influential variables: elevation, slope, aspect, presence of water, accumulation of surface water resources, and the distance to a point of water discharge (Montoya and Mesón, 1993; Duniway, 1983). The topographic water index (TWI) was used as an indicator of the accumulation of surface water resources. TWI was calculated using slope, flow direction and flow accumulation data obtained from ArcGIS software (directly whit hydrology toolbox that we can found in ArcGIS), scaled to a map cell size of 5.5meters. Results were standardized to values between 0 and 1. This method has been used according to Hjerdt *et al* (2004). Many authors have described that terrain soaking of during large periods favors pathogen proliferation, such as that of *Phytophthora cinnamomi* (Montoya and Mesón, 1993; Duniway, 1983). For this reason, the differential elevation between the individual examined and the closest fluvial course was calculated, in order to estimate the proximity of the tree to the phreatic layer and use this value as an indicator of potential soaking.

Older individuals are at higher risk of developing *la seca* disease (Montoya, 1981). Thus, the tree trunk perimeter was measured at breast height as a surrogate for the age. Finally, and according to Castillo and Acedo's (2013) categorization, the tree cover of susceptible species (*Quercus ilex* subsp. *ballota, Halimium umbellatum* sp. *viscosum* and *Cistus ladanifer*), resistant species (*Daphne gnidium, Helychrysum stoechas, Lavandula stoechas*), and indeterminate species (*Pinus pinea* and *Cytisus scoparius*) were identified and estimated within a square of 20 meters centered on the selected oak.

Data analysis was carried out using a general linear model (GLM), thus establishing a prediction model for the presence of *la seca*, as a function of environmental variables that were measured and standardised. A logistic regression was used to process binary data. The response variable was defined as healthy/unhealthy, in line with the aim of the study that was to detect the environmental variables involved in the distribution of the disease. The Akaike information criterion (AIC) was applied in order to select the best model (AIC takes into account how well a model adjusts to the observed data series, and the number of parameters used for the adjustment)

The environmental variables measured were involved into a binomial logistic model. Thereafter, the best model was selected by means of the function «*step, direction = both*» using RStudio 3.0.3 software. Residuals were tested for normality observing the frequency histogram, using the Shapiro-Wilk test, and by graphic means (g-g plot). Finally, we generated effect plots for each variable in the model.

RESULTS

For the selected model, the AIC value is 89.68 and $D^2 = 0{,}437$. Environmental variables that influence the disease ($p < 0.05$) are: difference in altitude a.m.s.l. between the affected holm oak affected and the fluvial course, topographic water index, cover of *Daphne gnidium*, cover of *Quercus ilex* subsp. *ballota*, and the trunk diameter at breast height. Only these variables have been used in the statistical model (Table 1).

COEFFICIENTS:	ESTIMATE	STD. ERROR	Z VALUE	PR(>|Z|)
(Intercept)	0.03759	0.32402	0.116	0.907633
Distance to the affected individual (m)	-0.53943	0.33114	-1.629	0.103313
Altitude difference (m)	-0.73965	0.32611	-2.268	0.023322*
Diameter (cm)	1.53512	0.42762	3.592	0.000328***
Coverage of *Quercus ilex* subsp. *ballota* (%)	0.86905	0.34409	2.526	0.011549*
Coverage of *Daphne gnidium* (%)	-1.19783	0.59157	-2.025	0.042886*
TWI	0.87197	0.43322	2.013	0.044138*

TABLE 1. *ENVIRONMENTAL VARIABLES* THAT SHOW STATISTICALLY SIGNIFICANT DIFFERENCES, THEIR P VALUES AFTER SUMMARY OF THE BINOMIAL LOGISTIC REGRESSION MODEL, AND THEIR COEFFICIENT VALUES.

Both, the model residuals histogram plot (figure 4.A) and the probabilistic graph for normality (figure 4.B) show adjustment to normality. Shapiro-Wilk test result is p=0.1395.

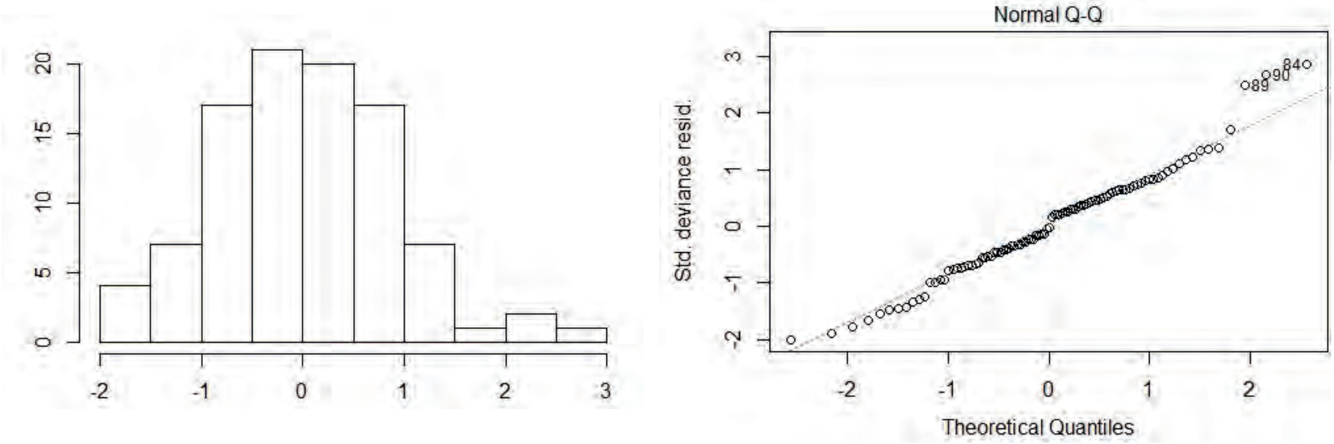

FIGURE 4. **A)** MODEL RESIDUALS HISTOGRAM PLOT. **B)** PROBABILISTIC GRAPH FOR NORMALITY.

Most of selected variables, except for the distance to the closest affected individual, have a significant effect on the probability of a tree being affected by *la seca*.

The altitude difference between the individual and the fluvial course is statistically significant and shows a negative effect (figure 5.A), thus showing that trees affected are in proximity to level of water. Hence, the concentration of water resource and the soil humidity are relevant variables in the distribution of the disease.

The age of the individuals (as related to the trunk diameter at breast height) shows a positive effect (figure 5.B): trees with a larger trunk diameter, and thus older, show higher probability of being influenced.

With regards to the several accompanying plant species, the presence of neither *Cistus ladanifer*, *Cytisus scoparius* nor *Pinus pinea* resulted in values other than 0. Their presence had no effect on the disease. However, we found differences related whit the cover of *Quercus ilex* subsp. *ballota*, with a positive coefficient value (Table 1), thus indicating that the degree of cover of this species increases the presence of affected holm oaks (figure 5.C). On the other hand, the presence of *Daphne gnidium* shows a negative value, and therefore a reduced risk of *la seca* disease (figure 5.D). Tree and shrub covers did not show statistical significance and therefore have no effect on the response variable.

Finally, the TWI shows positive significance, hence that areas with a higher index value, and thus higher humidity, are at a higher risk of *la seca* disease. (Figure 5.E)

DISCUSSION

Most studies performed in Spain that analyse the oak decline disease in the *Quercus* genus, suggest that summer temperatures causal factor for the *seca*. However, our research in Monte Valdelatas, as well as performed in different locations, establish that *la seca* is a disease where multiple environmental factors are involved

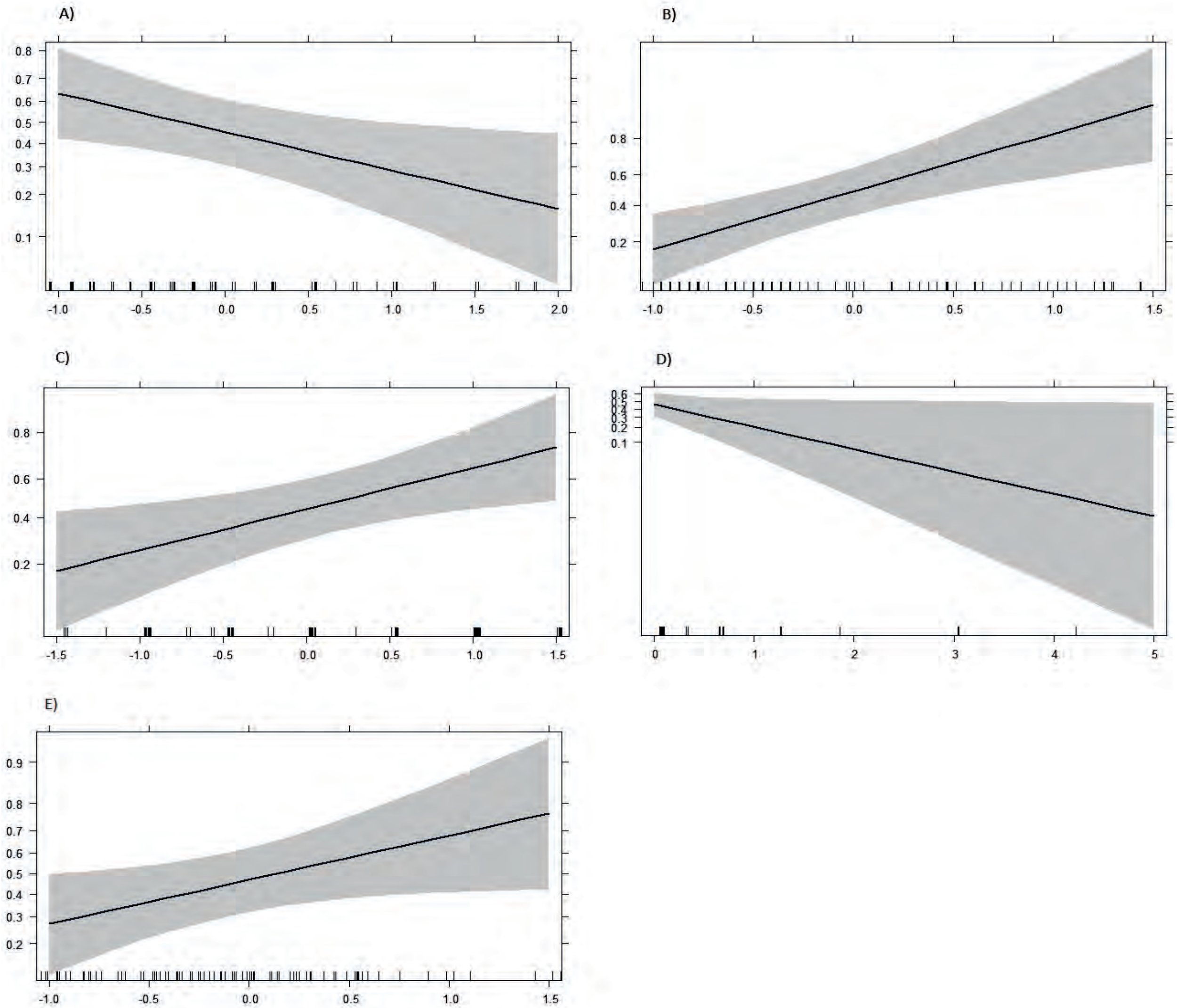

FIGURE 5. GRAPHIC REPRESENTATION OF THE ENVIRONMENTAL VARIABLES (X AXIS) AGAINST THE PROBABILITY OF PRESENCE OF *LA SECA* DISEASE IN THE TREE (Y AXIS). A) EFFECT OF THE DISTANCE TO LEVEL OF WATER (ALTITUDE DIFFERENCE (M) FROM THE INDIVIDUAL TO THE CLOSEST FLUVIAL COURSE). IT RELATES TO THE PROXIMITY OF THE INDIVIDUAL TO THE WATER LEVEL. B) EFFECT OF THE DIAMETER SIZE AT BREAST HEIGHT (CM). C) EFFECT OF THE COVERAGE (%) OF *QUERCUS ILEX* SUBSP. *BALLOTA*. D) EFFECT OF THE COVERAGE (%) OF *DAPHNE GNIDIUM*. E) EFFECT OF TWI.

(topography, water resources, soil texture and composition, tree age, vegetation composition and cover), that lead to a devitalization process that ultimately results in the death of the affected individuals. The factors involved have a great role in the development and propagation of the disease.

Since our results, the higher value of the trunk diameter for the holm oaks that show symptoms, corroborates the hypothesis, already mentioned in previous researches, that aged individuals are more vulnerable to the disease (Montoya and Mesón, 1994).

Results also show that among the predisposing factors (Montoya and Mesón 1994), the presence of certain species in the forest vegetation can favor or inhibit pathogen dispersion. Whilst *Cistus ladanifer* or *Cytisus scoparius* did not differentiate between zones in our study, the presence of *Daphne gnidium* showed a negative effect on the probability of *la seca* disease. The same trend is observed in a research by Moreira and Martins (2005). This shrub species has been traditionally used in ethnobotany for its toxics (coumarins and flavonoids) that present properties such as healing, vasodilatation, hypnotic, anti inflammatory, etc. In agriculture it has drawn attention for its antifungal properties, mainly due to the flavonoids: phenolic compounds (Phytoalexinas) that are generated on the cell surrounding and reinforce the cellular wall, therefore inhibiting pathogen hyphae growth (Cabrera and García, 1981). In terms of the results obtained, and the antifungal capacity of *Daphne gnidium*, it can be concluded that the presence of this shrub species in the holm oak stand, could hinder the appearance of *la seca* disease, related to *Phytophthora cinnamomi* Rands. On the other hand, a high holm oak tree density could act providing a higher number of individuals to host the pathogen and, thus, showing a higher probability of contagion.

Taking into account the development and colonization process of *Phytophthora cinnamomi* Rands and the need for free water for its movement (Tuset *et al*., 1996), it is to be expected that the proximity between affected trees allows for greater proliferation, where the pathogen could move from one host to another (Montoya and Meson, 1993). However, in the case of Monte Valdelatas, no significant differences were found when comparing distances between healthy-affected and affected-affected individuals. This situation might be due to the slope that impedes temporary water storage, a factor that contributes to the development of *Phytophthora cinnamomi* Rands and the colonization of new root tissue.

The presence of hydromorphic soils is one of the fundamental factors reflecting right conditions for pathogen development (Montoya and Mesón, 1994). In Monte Valdelatas, the presence of Arkose impedes water accumulation in the soil for long periods. On steep slopes, the water moves by surface runoff to depressed areas where it can be stored. These topographic depressions have a higher proportion of impervious material (gravels, clays…), avoiding seepage. These temporary water stores usually reach the plant root systems thus favoring the affection by fungi (Brasier *et al*., 1993). This explains the positive effect found for the TWI in the sense that higher value of TWI and higher capacity of temporary water retention lead to a significant increase of the disease affection.

It is worth noting that Hydromorphism is not always generated by means of surface runoff: it can also be due to the proximity to the phreatic layer. Such proximity increases the probability of contagion. This corroborates the idea that the proximity to fluvial courses intensifies the dispersion capability of the fungus, as water is retained for long periods in the soil.

Finally, it has been established that *la seca* disease may be artificially favored by irrigation management, water leaks, storage of water resources, etc. (Montoya *et al.,* 1992; Mesón and Montoya, 1997). It must be highlighted the presence in Monte Valdelatas of one artificial fluvial course generated by the leakage of residual water. On the field, we ascertained that in the surroundings of this artificial fluvial course, holm oak trees presented a higher degree of affection, and the results obtained coincide with this observation. It is interesting, so more studies are being done in relation of this residual water course.

Once the importance of environmental variables is established, the pathogenicity –as defined by the ability of an organism to produce toxins, colonise host tissue, and infect other individuals (Cardillo and Acedo, 2013)– takes on great importance. *Phytophthora cinnamomi* Rands fungus is considered to be responsible for most foci of *la seca*.

Current forest decline and the risk of further decline in Mediterranean holm oak and cork oak forest, amongst others species, highlights the need for buffering silvicultural managements designed to that reinforce the resistance capacity of *Quercus.* At the same time, it highlights the importance of acquiring knowledge about the environment in which the disease develops. By understanding the factors that support its development, mitigation mechanisms for the short and medium terms could be implemented as the creation of new forest patches, the progressive replacement of dead or affected trees by healthy individuals grown from controlled seeds, or the application of phytosanitary measures.

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