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Forest Ecology and Management 216 (2005) 166–174

Forest Ecology and Management

www.elsevier.com/locate/foreco

The relationship between *Cerambyx* spp. damage and subsequent *Biscogniauxia mediterranum* infection on *Quercus suber* forests

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Received 28 June 2004; received in revised form 5 April 2005; accepted 10 May 2005

Abstract

In this paper we shall show the first data on the level of *Cerambyx* spp. beetle damage and the subsequent *Biscogniauxia mediterranum* (De Not) Kunze fungus infection in the Extremadura region (southwestern Spain). Our results indicate a strong correlation between damage, caused by bad cork removal and pruning practices (injuries that cannot heal and permit the attack of insects), and holes made by beetles ($r = 0.86$). The relationship between holes and subsequent *Biscogniauxia* infection was $r = 0.91$. Different regression models have been proposed in order to find mathematical equations applied to forest health monitoring of wide areas. Additionally, we show the evolution in infection after 14 years in the four different areas. The results indicate an increase in damage by *Cerambyx* and *Biscogniauxia* infection after this period. Significant differences were found when comparing the four areas suggesting that local factors can be affecting these pests. Finally, we discuss the possible influence of different sociological, economic and agricultural factors on the management and conservation of *Quercus suber* forests in southwestern Spain and we propose possible solutions to integrate biological conservation and economic benefits of a sustainable cork industry associated with these Mediterranean forests.

Keywords: *Quercus suber*; Spain; *Cerambyx*; *Biscogniauxia mediterranum*

1. Introduction

Quercus forests (*Quercus rotundifolia*, *Quercus suber*, *Q. faginea* and *Q. pyrenaica*) of the Extremadura region (southwestern Spain) are agroecological systems similar to savanna, and characterized by the presence of livestock and/or wild species. These forests are commonly named dehesa and cover approximately 5,800,000 ha in western and southwestern Spain where they represent 52% of useful agrarian surface (Díaz et al., 1997). The dehesa is one of the last examples of traditional rural agroecological systems in Europe (Fabbio et al., 2003; Joffre et al., 1999; Morillo and Gómez, 2000). Nevertheless, the survival of the dehesa is threatened by different environmental problems. One of them is overgrazing by livestock (Plieninger et al., 2004; Leiva and

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doi:10.1016/j.foreco.2005.05.027

Fernández, 2003; Pulido et al., 2001, 2003). Together with livestock husbandry, the dehesa has been exploited for pruning in a clear model of multiple uses, thus, maintaining its ecological equilibrium and adaption to the sociological and economic framework of Mediterranean areas of Southern Europe (Scarascia-Mugnozza et al., 2000; Schmitz et al., 2003). From a methodological point of view, it has been demonstrated that this multi purpose management contributes to increasing plant and animal biodiversity in forests (Piussi and Farrell, 2000). These strategies of management are necessary in Mediterranean areas since *Quercus* species present a very low growth rate, a strong seasonality and they are not physiologically adapted to an intensive pruning (Baldocchi et al., 2004). In fact, the increase in the last decades of intensive management practices in Mediterranean forests, is producing a strong decrease in their regeneration and a parallel increase in their vulnerability to diseases (Piussi and Farrell, 2000). More specifically, *Q. suber* forest management in southwestern Spain, includes at least two treatments: pruning (Cañellas and Montero, 2002) and cork removal (Barberis et al., 2003; Borges et al., 1997). Nevertheless, cork removal is the main use of this tree species, mainly due to the low nutritive value, palatability and productivity of *Q. suber* acorns, in contrast to *Q. rotundifolia*, together with the high price of cork in the present European markets. Recently, different mathematical models have been developed to calculate the exact cork yield in the dehesa (Costa et al., 2001, 2003; Fonseca and Parresol, 2001). In the Extremadura region cork oak forests cover 252,140 and 600,000 ha mixed with other species. The cork related production in the last years varied from 18 500 Tm (1999) to 24 000 Tm (2003), this figure represents about a quarter of the Spanish production depending on the year. In 2003, the cork sector employed 1300 workers in the forest duties during 2 months with an average daily salary of 80€. In 2003 to the local cork production were added 18,000 Tm imported from other Spanish and Portuguese regions and 6500 Tm were exported without any transformation. The average non-manipulated cork price in 2003 was 2€/kg. After the first industrial treatment the cork price increased to 6€/kg and during this year 24,000 Tm were transformed. Secondary processes or cork for taps, agglomerates and similar products increase the prices per kg but these prices can vary according to the quality of the cork extracted in any particular year. These manufactured products were 24,000 Tm in 2003 for the Extremadura region.

Present costs of the cork removal process due to the high salary of specialized workers and the costs of chemical treatment in the industry has produced an important decrease in the profit margins of this traditional management (Benitez et al., 2003).

According to Fialho et al. (2001) cork removal does not affect the radial growth rate but has a slight effect on leaf abscission. Nevertheless, a bad cork removal process impedes the natural healing of injuries and facilitates the development of diseases (Montoya Oliver, 1988). A phenomenon with a high incidence in the cork oak forests of Sierra of Jerez is the attack of *Cerambyx* beetles. This environmental pest affects a large number of trees and forest areas. The life cycle of *Cerambyx cerdo* (El Antry, 1999) begins with larvae development that is produced 10 days after egg-laying. In the next 28 months this species has five larval instars. According to Montoya Oliver (1988) the larval stage increases in size up to approximately 70 mm in length and 16 mm wide. Adults remain inactive for a further 7 months until spring when they emerge to mate. The average longevity of the imago is 13 days and the maximum fecundity reaches approximately 305 eggs. Exit holes produced by *Cerambyx* larvae are one of the entryways of fungal infection by *Biscogniauxia mediterranum*. Under natural conditions, this fungus disease attacks decrepit *Quercus* trees or trees that suffer damage of a meteorological or biotic origin. In managed forests, the fungus spore are transported by pruning or cork removal forestry equipment infecting other trees. This fungus has a very slow growth and remains in the tree until the infection is very advanced. Then it produces the spore and the typical charcoal black surface in the branches and trunks then appears. In healthy trees with a low level of injuries a certain resistance can be shown and the progression of the disease stops. In other cases, charcoal disease can kill the tree (Montoya Oliver, 1988).

According to European Directives for the preservation of the natural environment (Habitat Directive, 1992, Annex I,) the oak and holm-oak forests are protected in Europe. Therefore, the pest control of *Cerambyx* spp. could be a desirable management strategy. Nevertheless, some species of the Cerambyx genus such as *Cerambyx cerdo* are protected by the same European Directive (Habitat Directive, Annex IV, 1992). In the present work, we analyze the effect of pruning and cork removal on *Cerambyx* spp. damage and *B. mediterranum* (De Not) Kunze (Mazzaglia et al., 2001) infection. Our results have enabled us to reach a clear dramatic conclusion: the present management practices on *Q. suber* forests in southwestern Spain are producing a very strong environmental impact that could threaten their conservation and regeneration within a few decades.

2. Material and methods

2.1. Sampling sites

Four transects with a dominance of *Q. suber* or mixed *Q. suber* and *Q. rotundifolia* forests in southwestern Spain (Sierra de Jerez, Extremadura region) were sampled in 2 different years (1989 and 2003) in order to test the evolution of fungus infection (Fig. 1). These transects were located by using a 1:50,000 topographic map (Servicio Geográfico del and Ejercito, 1959). In each transect different permanent plots with similar conditions of soils, forest densities and slopes were selected to avoid possible micro climatic differences (Auslander et al., 2003).

The climatology of the Sierra de Jerez is semiarid Mediterranean with an average rainfall 490–800 mm, average temperature of 15 °C and a drought period of 4 months. The cork trees cover 100,000 ha, pure and mixed in this area. The trunk-diameter classes show a different distribution in the four transects analyzed (Fig. 2). Every 9 years the trees underwent cork removal as usual in the forestry management in this area. According to Guerra Delgado et al. (1968) the characteristics of the soils of Sierra de Jerez are eutric cambisol (FAO, 1998) characterized by a scarce A horizon with low humic content and a B horizon with brown color due to partial decomposition of organic matter and mixed with mineral material. The texture is franco-arenosa and the pH is close to 6. The organic matter contents oscillates between 1 and 2%. The cationic interchange capacity is between 15 and

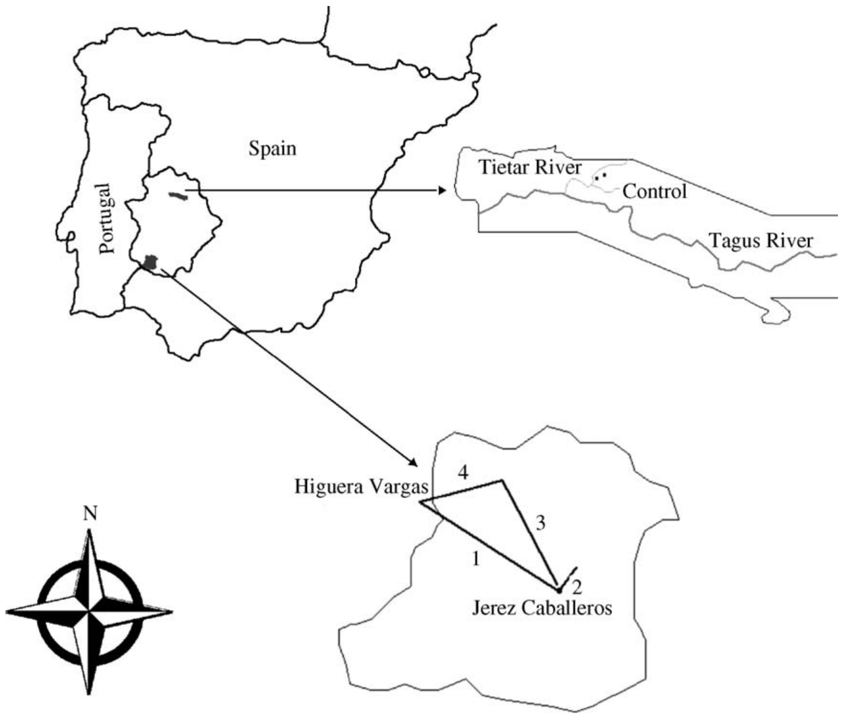

Fig. 1. Location of sampling areas in Extremadura region (southwestern Spain).

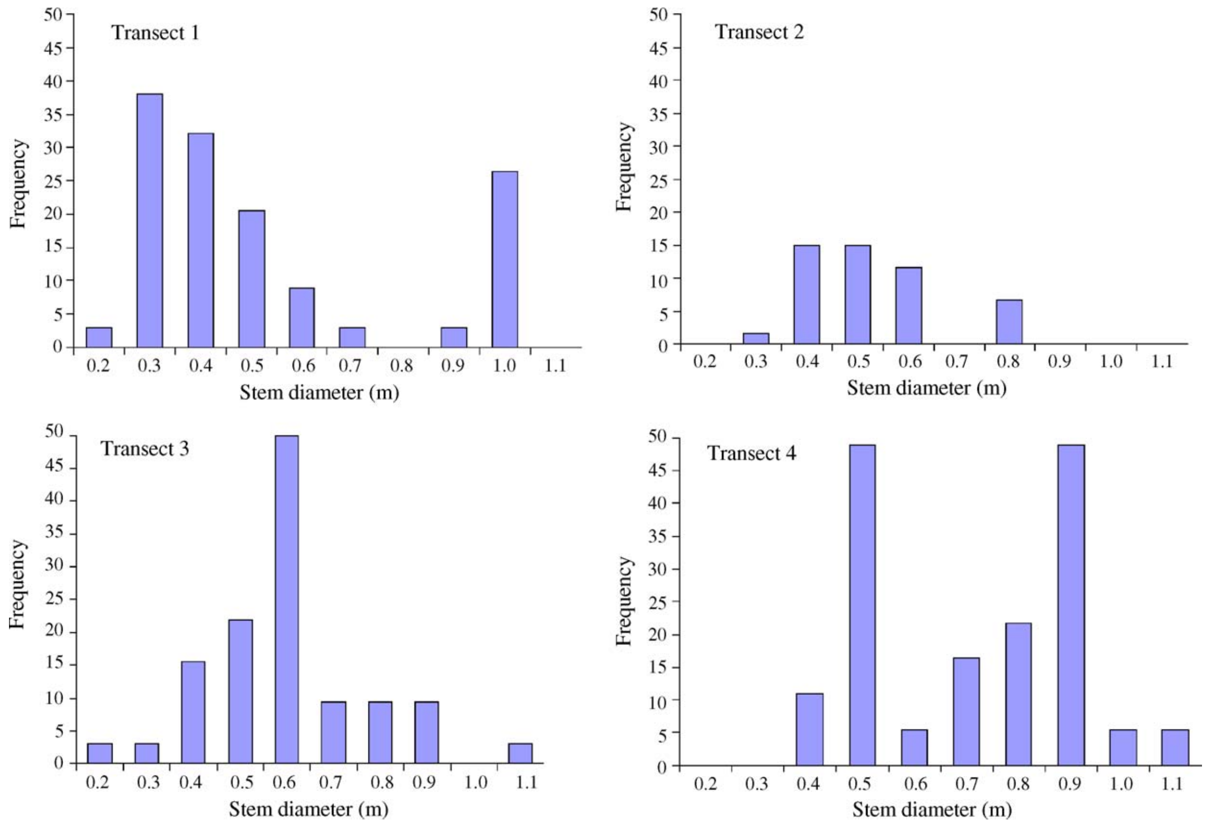

Fig. 2. Frequency of trunk diameter classesin the four transects analyzed.

25 cmol kg^{-1} and the degree of water saturation is close to 60%. The formation of this soil type is influenced by the original acid material, the low rainfall and high summer temperatures that reduce the annual period of decomposition of organic matter. This soil is the considered climax for *Quercus* spp. forest under Mediterranean climate.

As the control group, a 100 tree sample from two different permanent plots selected in two adjacent sites inside protected areas of Monfragüe Natural Park (between 39°45′ and 40°00′ latitude N and 5°45′ and 6°00′ longitude W) (Fig. 1), was used. The Park covers 17,852 ha mainly of Mediterranean forests and shrublands and is the only place in the Extremadura region with cork oaks not used under forestry management (cork removal and/or pruning). The control trees from Monfragüe Natural Park were practically not pruned or underwent cork removal in the last 25 years. Obviously, as these characteristics are very unusual, it was not possible to locate a closer control group to the rest of the forest under management. Nevertheless, we consider that this choice was appropriate due to the high differences in infection between the control and sampling areas, which cover a large extension with a large number of trees analyzed. The average altitude is 360 m with an average annual rainfall of 791 mm corresponding to a sub-humid Mediterranean climate (Rivas-Martínez, 1987). Nevertheless, the rainfall is very unevenly distributed due to the 6-month period of drought (from May to October) in the driest years. The soils are haplic acrisol characterized by B stratum with cationic interchange capacity of 24 cmol kg^{-1} and a degree of water saturation of 50% (Devesa, 1995).

The botanical composition from the two studied areas, the Sierra of Jerez and the control area in Monfragüe Natural Park are shown in Table 1.

Table 1
Botanic composition (families, number of species and abundance) characteristics of Sierra de Jerez and Monfragüe Natural Park

Families	*N* species		Abundance (%)	
	Sierra Jerez	Monfragüe	Sierra Jerez	Monfragüe
Anacardiaceae	2	2	28.30	6.57
Araliaceae	1	0	7.60	0.00
Asteraceae	1	0	14.10	0.00
Caprifoliaceae	2	2	21.20	20.82
Cistaceae	7	8	85.20	37.96
Cupressaceae	0	1	0.00	1.57
Ericaceae	1	3	7.80	16.78
Fabaceae	7	5	28.50	9.95
Fagaceae	3	3	35.30	9.21
Lamiaceae	6	4	28.40	20.20
Liliaceae	2	2	14.10	2.09
Myrtaceae	0	2	0.00	5.00
Oleaceae	1	4	21.30	15.25
Pinaceae	0	1	0.00	1.50
Rhamnaceae	0	1	0.00	3.00
Rosaceae	5	4	14.90	3.35
Rubiaceae	0	1	0.00	3.22
Santalaceae	1	1	21.80	2.60
Smilaceae	1	0	7.20	0.00
Thymelaceae	1	1	35.30	3.85
Ulmaceae	0	1	0.00	1.00

2.2. Sampling method

The average diameter, measured at 1.3 m, of a batch of trees in each of four transects was registered (Table 2 and Fig. 2). In each tree, age, the presence of cork removal and/or pruning damage, the holes of *Cerambyx* and the presence of *B. mediterranum* were measured. Age was divided in three categories: juveniles (between zero and two cork removals in their lives), adults (more than two cork removals and with trunk diameter less than 60 cm) and old trees (more than 60 cm of trunk diameter). These categories were created as a fast age estimation method in the field. According to Montoya Oliver (1988) decorking damage can be considered to be any cork removal that produces a break (cut) that exposes the wood. Within this category are grouped both axe hits and the separation of the phellogen by inadequate extractions. This author considered pruning damage to be injuries that the tree cannot heal, such as deep cuts in the bark, cuts with rips and inclined cuts where water is trapped. In this paper we recorded damage, both by pruning or decorking, when its size was bigger than 20 cm of diameter. This diameter is the maximum pruning size allowed according to the Regional Government's Dehesa Law and the National Forest Management Law (Ley sobre la dehesa en and Extremadura, 1986). In the Sierra of Jerez shape pruning is focused on acorn production. This produces an architecture of trees characterized by a short trunk, low main branches and a big canopy. Maintainance pruning usually has been abusive and frequently big branches have been cut. As a consequence the trees show a defective growth habit and premature aging (Montoya Oliver, 1988). *Cerambyx* produces holes in trunks when the imago finishes its larval development and emerges from the tree. A semi-quantitative scale of holes for a fast counting in the country was carried out. This scale was divided into five categories: zero (no holes), one (from one to three holes), two (from four to ten) and three (more than ten). *Biscogniauxia* damage was registered as the presence or absence of charcoal disease.

Three different observers evaluated independently the variables in the infection of each tree. The final calculations were the average of the three observers. The tree species analyzed was cork oak (*Q. suber*) and the total number of trees was 725 in 1989 and 834 in 2003.

2.3. Statistical analysis

Unmanaged (control) and managed forest were compared in the amount of damage, the number of

Table 2
Characteristics of *Quercus suber* forest in the four sampled transects

Variable	Transect			
	1	2	3	4
Stem diameter (m)	0.68 ± 0.30	0.77 ± 0.18	0.89 ± 0.25	1.05 ± 0.31
Density (trees/ha)	27.65 ± 7.14	26.66 ± 3.73	63.38 ± 39.46	79.34 ± 48.45
Injuried trees (%)	47.27 ± 12.45	36.07 ± 18.16	31.23 ± 18.50	42.67 ± 22.75
Stand purity (% trees of *Quercus suber*)	58.89 ± 21.47	99.81 ± 1.3	94.75 ± 5.5	75.71 ± 35.99

holes (grouped in the above four categories) by *Cerambyx* and *Biscogniauxia* infection using a Mann–Whitney non-parametric test (Sokal and Rohlf, 1984). Inside the managed forests, the categories of holes of *Cerambyx* and the presence/absence of charcoal disease were tested according to site, year and interaction of both factors by using the Kruskall–Wallis non-parametric variance test (Sokal and Rohlf, 1984). Averages between six sites for 2 years were calculated to avoid possible inter-tree variabilities independent of management factors. These data were used in two regressions between the following pairs of factors: level of damage-number of holes (grouped in categories) and number of holes—*Biscogniauxia* infection. Regressions were tested in R2, ANOVA tests, normality of residuals and homoscedasticity (Montgomery and Peck, 1989). The number of possible outliers was also calculated (Jobson, 1991). The comparison between age and level of *Cerambyx* infection, both in trunks and branches, was analyzed by means a non-parametric Kruskal–Wallis variance test. In all the analyses the R statistical package under a Debian GNU Linux workstation was used (R Development Core and Team, 2003).

3. Results

In a first analysis, considering all the trees as a group, we obtain significant correlations between damage and holes due to *Cerambyx* attack ($r = 0.86$) and holes and subsequent *Biscogniauxia* infection ($r = 0.91$). A more detailed linear regression analysis applied to these same factors showed significant relationships between them (Fig. 3); both regressions show high R^2, with significant ANOVA tests, low standard error of estimation, non significant heteroscedasticity, normality of residuals and with a low number of outliers (Table 3).

When the age of trees (in classes) was considered separately, the differences between levels of infection of *Cerambyx* (in categories) was statistically significant in the 2 years of sampling both in trunks and branches (Table 4).

When we consider the differences in the *Cerambyx* holes for the four transects, it is clear that the infection increased from 1989 to 2003 (Fig. 4). The analysis of the effect of both factors separately and in interaction

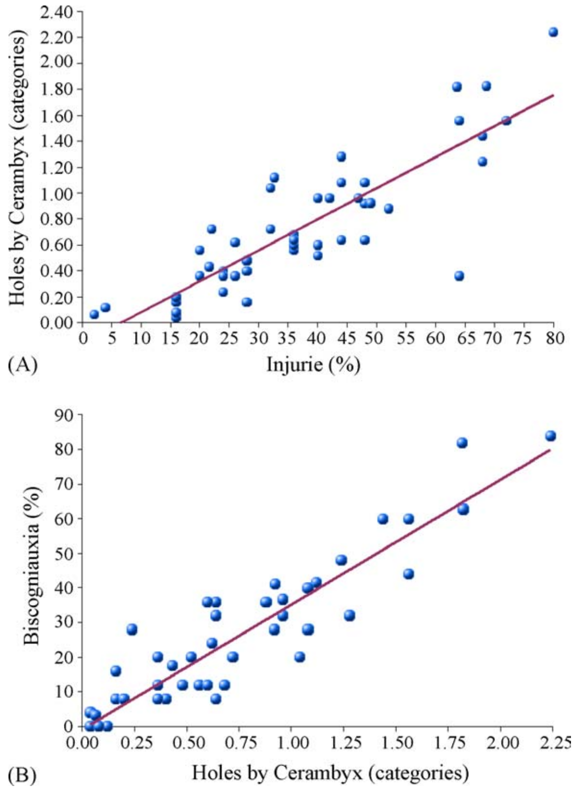

Fig. 3. Linear regressions between: (A) *Cerambyx*'s holes and injuries caused by bad management of *Quercus suber* trees and (B) *Biscogniauxia* infection related to *Cerambyx's* holes.

showed significant differences except for the effect of year in trunks (Table 5).

Differences between years were significant in *Cerambyx* holes and *Biscogniauxia* infections (Table 6). Only the differences between transects in charcoal disease were not significant. Nevertheless, when the combined effect of transects and years was analyzed, the differences were significant both for *Cerambyx* holes and charcoal disease.

The comparison between the control group without management (Monfragüe Natural Park) and the four transects in the Sierra de Jerez shows significant differences, for injuries ($W = 20109$, $p = 3.59\text{e}{-10}$), holes of *Cerambyx* ($W = 22952$, $p = 9.378\text{e}{-11}$) and *Biscogniauxia* infection ($W = 22278.5$, $p = 6.72\text{e}{-12}$). The control group showed a very low level of holes (0.24 ± 1.53) and charcoal disease (0.03 ± 0.18).

Table 3
Main statistics of both regression models

Model	Holes–damage	*Biscogniauxia*–holes
Equation	Holes = 0.02 × damage	*Biscogniauxia* = 35.208 × holes
R^2	$R^2 = 0.93$	$R^2 = 0.94$
ANOVA	$F = 587.4$ ($p < 2.2E-16$)	$F = 695.9$ ($p < 2.2E-16$)
Standard error of estimation	see = 0.21	see = 8.24
Heteroscedasticity	bp = 0.0021 ns	bp = 0.4004 ns
Normality	$W = 0.963$; $p = 0.169$	$W = 0.984$, $p = 0.7478$
Outliers (%)	8.33	0.00

W: Shapiro's test of residuals normality; bp: Breusch-Pagan test of homoscedasticity; ns: non-significant statistical differences.

4. Discussion

The results obtained show that is perfectly possible to consider that damage caused by inappropriate management is directly the prime cause of the increase in holes made by *Cerambyx* beetles. In addition, these holes caused the subsequent *Biscogniauxia* infection in a two-step chain of events. The regression obtained could be used for reliable predictions in future studies or for management purposes. Furthermore, forestry managers could predict the possible levels of *Cerambyx* holes and charcoal diseases expected in non-correctly managed forests due to injuries causes by bad pruning and/or decorking. With additional information in future studies related to the expected mortality of trees, the equations obtained could be used to determine the maximum allowed damage in forests.

Typically, *Cerambyx* is an insect linked to the natural senescence (aging) of trees, but inappropriate pruning or decorking activities increase the stress in trees causing a beneficial insect to become a very important cork-oak forest pest. The same considerations can be applied to *Biscogniauxia* infection (Gallego et al., 1999; Luque et al., 1999; Oliva

Table 4
Relationship between year and age classesand *Cerambyx*'s holes in branch and trunks of *Quercus suber*

Year 1989	Branch	Trunk
Class 1	0.048 ± 0.378	0.095 ± 0.530
Class 2	0.691 ± 1.250	0.245 ± 0.811
Class 3	1.413 ± 1.478	0.919 ± 1.377
Test	$X^2 = 69.668$***	$X^2 = 68.063$***
Year 2003	**Branch**	**Trunk**
Class 1	0.000 ± 0.000	0.000 ± 0.000
Class 2	0.838 ± 1.334	0.485 ± 1.086
Class 3	1.325 ± 1.455	1.052 ± 1.440
Test	$X^2 = 14.787$***	$X^2 = 12.906$***

Results of Kruskal–Wallis (KW) test are shown.
*** p-value <0.001.

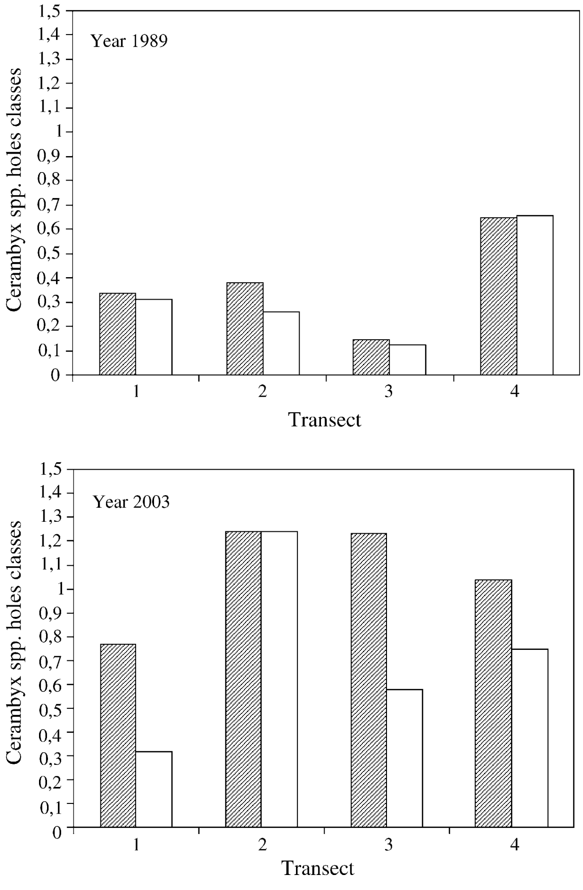

Fig. 4. Changes between 1989 and 2003 in the averages of *Cerambyx* spp. holes (classes). Effects on branches (shadow bars) and trunks (white bars) are shown.

Table 5
Comparison of level of holes produced by *Cerambyx* spp. in trunks and branches considering the effects of transects, year and the interaction between them

Variable	Factors	KW test
Holes in trunks	Site	19.31; $p = 2.35E{-4}^{***}$
	Year	3.61; $p = 0.057$ ns
	Site × Year	26.65; $p = 3.86E{-4}^{***}$
Holes in branches	Site	7.85; $p = 0.049^{*}$
	Year	17.32; $p = 3.15E{-5}^{***}$
	Site × Year	28.68; $p = 1.66E{-4}^{***}$

Results of Kruskal–Wallis (KW) test are shown; ns, non-significant differences.
* p-value <0.05.
*** p-value <0.001.

Table 6
Effect of Site, Year and both on level of holes produced by *Cerambyx* spp. beetles (upper side) and presence of charcoal disease (lower side) in cork-oak forests

Variable	Factors	KW test
Number of holes	Site	11.92; $p = 0.017^{*}$
	Year	11.54; $p = 6.81E{-4}^{***}$
	Site × Year	30.27; $p = 1.28E{-4}^{***}$
Charcoal disease	Site	8.15; $p = 0.086$ ns
	Year	16.35; $p = 5.26E{-5}^{***}$
	Site × Year	29.13; $p = 3.01E{-4}^{***}$

Results of Kruskal–Wallis (KW) test are shown; ns, non-significant differences.
* p-value <0.05.
*** p-value <0.001.

Estanyol and Molinass de Ferrer, 1984). In our results the incidence of *Cerambyx* and *Biscogniauxia* is not the same according the age of trees. In fact, in inappropriately managed forests the attack of these organisms affect even young trees.

The first registers of incidence of *Cerambyx* pests in the Extremadura region are from the 1980s, very close to our first data from 1989. In 14 years there has been a serious increase in the infection levels. If this tendency continues, the future of *Q. suber* forests in the area studied could be compromised.

The results in Monfragüe Natural Park are very clear. Unmanaged forests show very low levels of infection, which represents the levels expected in natural ecosystems. Nevertheless, it is impossible to leave all the cork-oak forests of the Extremadura region without any management, especially at present when the cork market is in recession (Cabecera, 2004). It may be possible to reach a certain low level of damage with a consequent low level of charcoal disease in this type of forest but future studies are necessary.

The effect of *Cerambyx* and *Biscogniauxia* on Mediterranean ecosystems could have a strong environmental impact, which, combined with other factors such as livestock pressure on the natural regeneration of propagules of *Quercus* forests (Pulido et al., 2001), or climatic change (Vannini et al., 1996) could compromise (negatively affect) the conservation of Mediterranean areas in the very near future.

From a practical point of view and in order to avoid the serious environmental impact that we have measured, we recommend the use of workers with experience in the forestry activities related to *Q. suber* exploitation, the extraction of cork with specialized technology, such as the IPLA machine (Antolín et al., 2003), the extension of technical courses and a strict regulation of cork removal in Mediterranean forests of the Extremadura region.

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