The decline of Cork oak (*Quercus suber*) and Holm oak (*Q. ilex/ rotundifolia*) in South Western Portugal

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1 Introduction

In Portugal 38% of the total land cover is stocked by forests. This figure can be raised to 64% if unproductive and wastelands are taken into consideration (ALVES, 2003). Although only 21 % of the forest area is dominated by Cork oaks , this species takes a very special position in the land, because with a 57% share Portugal clearly dominates the world cork-market (CARVALHO MENDES, 2002) which is quite an achievement for such a comparatively small country. 80% of the forest area is owned by private land owners (ALVES, 2003). Most of them are small farmers with an average farm size of less than 5 ha (BORGES, 2007, internet 1). The main territory occupied by *Quercus suber* lies in the hinterland and the drift towards the cities and land abandonment is a big problem in these areas with the structure of the remaining population containing a high percentage of older people (MOREIRA DA SILVA, 2003). As a result many properties lie fallow or are insufficiently managed and the possibilities of intervention by the state are very limited. In these areas cork production is an important economic and social factor, providing an income for the local population.

The classical management system of these oak stands is called “montado” and combines forestry with agrarian and especially pastoral land use patterns. This land use system is characterised by very extensive, as well as multifunctional attributes. Besides the cork production timber, fuel and charcoal are the main forestry products of the montado, but there are a lot of non-wood products such as honey, mushrooms and medical plants obtained as well. On the agricultural side the main use pattern is cattle-breeding underneath the trees, which protect from weather extremes, with the acorns adding a valuable supplementation to the pastures in the autumn. The social component is maintained by providing employment on the one hand. On the other, these woodlands are used for recreational activities, such as hunting, and- increasingly in recent years- tourism (CARVALHO MENDES, 2002). Moreover, these oak stands are rich ecosystems characterised by a high biodiversity and play an important role in combating desertification, which is a permanent threat to these semiarid regions (GIL, L., 2007, internet 2). In the last decades however, this old cultural landscape is afflicted by several new influences, such as

- Climate changes: prolonged summer drought and heavy winter rains.
- Cultural changes: Land abandonment and the consequent large scale monocultures, many of them with exotic species (e.g. *Eucalyptus globulus*), on traditional oak territories.

- Big wildfires: most of them with anthropogenic causes.
- A disease, the local farmers call “secca” (drought), which shall be referred to in the following work. (Modified according to ROSE, 2005)

The aims of this work are to survey a certain area affected by oak decline and to determine the causal agents. Further, the formation of the symptoms shall be examined and related to possible pathogens. It will be attempted to recognize a pattern for the infestation of trees in a schematical, as well as topographical respect. Finally, a set of recommendations for the technical treatment of infested areas shall be outlined.

Fig. 1 View over the hills from Corte Malhao, afforestation project at the front, cork oak montados at the back. T. Kaltenbach, 26.III. 2007

2 State of knowledge

2.1 Description of the tree species concerned

2.1.1 *Quercus suber* LINNÉ

Cork oak stands occur in descending order of size in Portugal, Spain,Algeria, Morocco, Italy, Tunisia and France (GIL, 2007, internet 2). The following description is based on the characterisation given by BUSSOTTI and BOTTACCI (1999):

Q. suber is an evergreen, middle- sized tree species of the western Mediterranean. Adult trees reach sizes of 15- 20 m, exceptionally 25 m. Cork is extracted every 9 years and a single tree can produce 100-200 kg of cork during its life time. The life- span depends on the intensity of cork extraction and varies between 200 years, for regularly harvested trees, to more than 400 years for those not harvested. The species is characterised by high demands for light and warmth and grows in an area with a mean temperature of 13- 17° C throughout the year. Maximum temperatures of up to 40° C are tolerated by temporary reduction of metabolism, minimum temperatures to – 10° C can be outlasted without major injuries. *Q. suber* is modest in regard to soil requirements. It is able to grow on poor sandy and clayey sites of granitic and schistic origin. The pH should lie within 4,5 and 7, however limy sites are avoided. The ideal amount of precipitate lies between 500 and 700 mm, but it can cope with less, sufficient air moisture presupposed. The ssp. *occidentalis* occurs only in Portugal, with an affinity to the Atlantic coastline and differs from the main species by a two year development cycle of the acorns and a semi-evergreen foliage. Cork oaks develop a tap root system, which can be subdivided into two layers. In the upper layer fine roots spread from the main tap directly below ground level in a depth of ~ 30 cm. The second layer can vary in depth and is formed in contact with the underlying mineral course (HARRACHI, 2000).

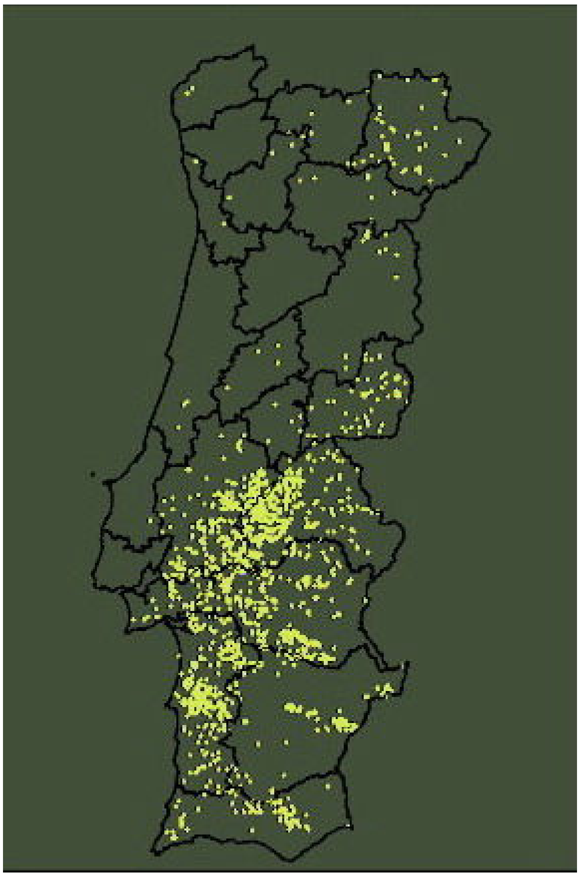

Fig. 2 Distribution of *Quercus suber* in Portugal
From: MINISTERIO DE AGRICULTURA, 2006

Q. suber in Portugal

Covering 736700 ha, *Q. suber* takes the first place by forest area, according to the latest Forest Inventory (DGRF, 2007) and is the main economic tree species in Portugal (MINISTRY OF AGRICULTURE, 1995). More than 70 % (527200 ha) of these stands are situated in the Alentejo region. The majority of these cork oak stands still originate from natural dissemination; plantations to a greater extent were only realized in the last few years (MINISTRY OF AGRICULTURE, 1995).

2.1.2 *Quercus ilex* LINNÉ/ *Q.rotundifolia* LAM.

The description of this species is based on the work of KNOPF (2002):

The holm oak is a rather small species of usually 8-10 metres, under favourable conditions up to 25 m in height. It has a very broad amplitude in regard to nutrient and water requirements. Sites can vary from silicate to chalk based substrate and extreme drought (~300 mm/ annum) to high moisture (~ 1200 mm/ annum). In the same way, the climate spectrum differs between atlantic- fresh to continental- hot, showing a temperature span between + 44° and – 24° C. However, a high amount of light is a precondition. It is ousted by the cork oak on the richer and wetter sites but has a wider amplitude of demands and consequently occupies a bigger area worldwide. Like the cork oak, it is an evergreen sclerophyte and is a climax- species in many semiarid north African and south European regions.

The wood is extremely hard and heavy, though elastic and is used for fuel, carpenters wood and even veneer. A very good charcoal can be obtained from it. The sapwood is indistinguishable from the core by colour. Very unusual for an oak is the scattered alignment of the pores. In some regions the bark is still used for tan.

The morphotype *rotundifolia* prevails on the Iberian peninsula and in north Africa. It is characterized by rather round shaped leaves and produces sweet acorns, which are commonly used for cattle feed , and even for human consumption.

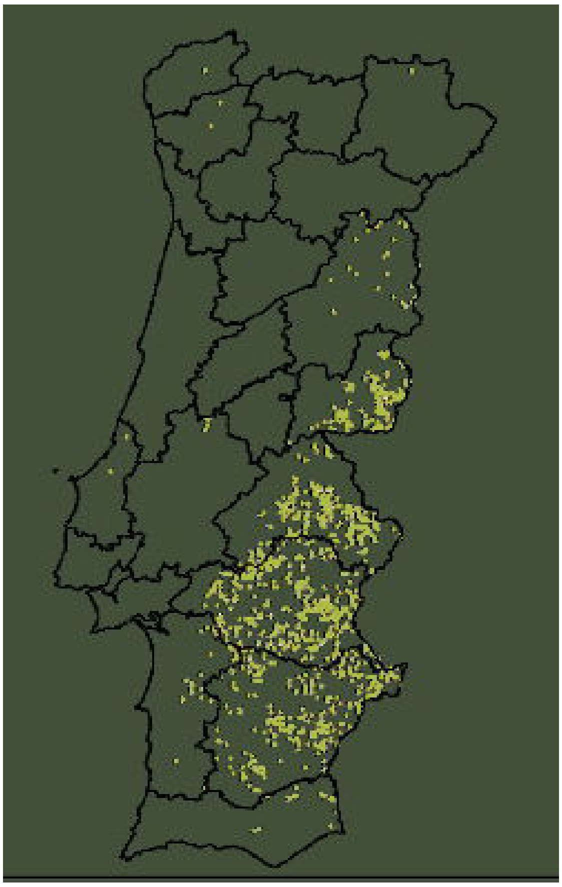

Fig. 3 Distribution of Quercus rotundifolia in Portugal From: Ministerio de Agricultura, 2006

Q. rotundifolia **in Portugal**

Like for the cork oak an emphasis of the distribution lies in the Alentejo region, but for the holm oak the natural centre lies further eastwards(ALVES, 2003). The holm oak holds the fourth place in terms of land cover with 388300 ha of surface. However, the trend is decreasing compared with the results of the last forest inventory (1995), when it still occupied 461600 ha (DGRF 2007). This means a loss of 16% in only 10 years. Reasons for this drastic decrease are subscribed to the abandonment of traditional farming and consequently a loss of economic interest in this species. Another factor in this context is the increasing oak mortality.

Although Portugal is a comparatively small country, environmental conditions vary a lot. As a result, both species find natural conditions close to their ecological limits in some areas. This leads to the formation of pockets of populations with unique genetic resources. Some of them are already extinct. As it is these resources that might be the most interesting in the context of climate change, a programme for the conservation of genetic resources was set up for *Quercus suber* in the 1990ies (MINISTRY OF AGRICULTURE, 1995; VARELA, 1995).

2.2 Oak decline in the Mediterranean region

Decline scenarios are reported from all over the territory occupied by *Quercus suber* and *Q. ilex/ rotundifolia*. From Italy RAGAZZI et al. (1997) portray a complex disease on several Oak species including *Q. suber* and *Q. ilex* with *Diplodia mutila* Fr. apud Mont. (telemorph: *Botryosphaeria stevensii* Shoemaker) taking a key role. VANNINI and MUGNOZZA (1991) describe *Biscogniauxia mediterranea* (previous: *Hypoxylon mediterraneum*) as an aggressive agent in drought- stressed *Quercus cerris* forests in Italy. An ink disease on *Quercus rubra* caused by *Phytophthora cinnamomi* Rands in south-western France was identified as early as 1952 (MOREAU and MOREAU, 1952 in ROBIN, 1992). The same disease is the basis for the studies of MARCAIS et al. (1993). A very extensive survey is in hand about the cork oak dieback in the Maamora Forest in Marocco. HARRACHI (2000) assumes a complex of causes, initiated by an extreme drought and accompanied by insect damage. He considers *Diplodia mulila* and *Biscogniauxia mediterranea* as secondary agents for the disease. In 1992 BRASIER (1992) suggests the involvement of *Phytophthora cinnamomi* in Iberian oak mortality, specifying his thesis in later essays (BRASIER et al. 1993; BRASIER, 1996). LUQUE and GIBRAL (1989) report cork oak dieback from Catalonia (NE Spain), caused by *Diplodia mutila.* FERNANDEZ- ESCOBAR et al. (1999) determine *Phytophthora cinnamomi* Rands to be involved in oak decline in western Spain by a selective fungicide method.

In Portugal oak decline has been described since the end of the 19th century (ALMEIDA, 1898; CAMARA- PESTANA 1898 in MOREIRA et al. 2006). In the context of *Phytophthora* it is interesting to know that the ink disease, prevailing on *Castanea sativa* since the 1940ies, is caused by *Phytophthora cambivora* (Petri) Buis and can infect *Quercus suber* as well (PIMENTEL, 1946). Three years later, PIMENTEL reports *P. cinnamomi* to be another agent of this ink disease (PIMENTEL, 1949, in BRASIER, 1996). In the National Forest Strategy, the Forest Directory (DGRF, 2006 a) states that an ongoing process of disease was noticed for the two oak species *Q. suber* and *Q. rotundifolia* since the 1970ies, particularly since 1997. As a direct result the total cork production in the 1990ies decreased 34% compared to the output still produced in the 1960ies. Figure 4 shows the increasing amount of diseased trees in percent with the highest rates for *Q. suber* and *Q. rotundifolia.*

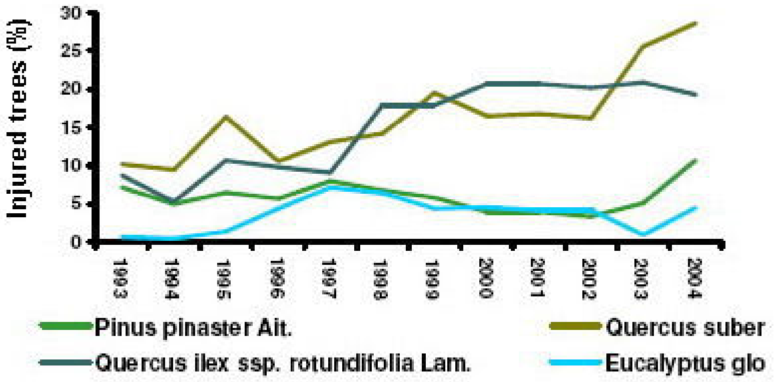

Fig. 4 Trees of low vitality from the most common species in Portugal
From: Estratégia Nacional para as Florestas, DGRF, 2006 a

After surveys in Portugal and Spain 1991/ 92 Brasier et al. (1993) propose *Phytophtora cinnamomi* to be a major contributory factor for the disease. Since then surveys have been undertaken on this task by several researchers (Moreira and Martins 2005; Moreira et al., 2006) and *P. cinnamomi* has been identified on many different sites throughout Iberia. The three fungal pathogens regularly referred to in the context of Mediterranean oak decline are *B. mediterranea, D. mutila* and *P. cinnamomi (* Harrachi 2000; Luque and Gibral, 1989; Brasier et al., 1993). Of these three *D. mutila* and B. *mediterranea* are usually referred to as opportunistic pathogens on predisposed hosts (Sinclair and Lyon, 2005; Sinclair et al, 1987). However Luque et al. (1999) carried out a survey on the pathogenity of the three and though the test groups were extremely small (only three plants per fungus), and in the case of *Diplodia* even one plant was excluded, designated *Diplodia* to be the most aggressive, followed by *Phytophtora*. *Biscogniauxia* was deemed to be the less virulent. The work of Harrachi (2000) showed similar results. In this work however, the main focus is set on *P. cinnamomi*, because it is the most aggressive of the pathogens found in the research area (Brasier et al., 1993).

2.3 Description of possible pathogens

2.3.1 *Phytophthora cinnamomi*

BRASIER (1996) considers *P. cinnamomi* as a primary pathogen with a very wide host range and one of the most destructive tree root diseases worldwide. He locates its origin in Papua New Guinea and dates its introduction to Europe in the early nineteenth century.

Procedure of the disease

BRASIER et al. (1993) set up a hypothesis, according to which two different types of disease procedure can be distinguished:

- A more rapid form, which is caused by substantial root death or stem girdling. Trees die in one or two seasons. This procedure can especially be observed on soils that remain moist throughout the year, such as valley bottoms.
- A more chronic decline, where a certain amount of fine root loss can be replaced each year. It occurs on only seasonally moist sites, e.g. slopes. In this case the trees are able to resist the disease for a while, until additional stress factors, such as extreme drought or secondary infections appear and induce death.

General symptoms

Several authors (MOREIRA and MARTINS, 2005; BRASIER et al. 1993; FERNANDEZ-ESCOBAR et al, 1999) describe the basic symptoms as follows: Chlorosis of leaves, reduction of leaf size and the occurrence of necrotic leaves are the first signs, followed by increasing defoliation and the appearance of dead branches. On some trees water sprouts are produced and tarry exudations can be observed on 5- 10 % of the infected trees. These exudations occur at the root base or lower trunk region and originate from a cambial necrosis that characteristically assumes a tongue- like shape beyond the bark (KEHR, 2004). Sudden wilting, stem girdling and rapid death within several weeks or months may prevail in the accelerated form. As *P. cinnamomi* is highly polyphagous, extensive patch dieback of shrubs around the affected trees may accompany the oak disease (BRASIER et al., 1993).

Classification

AGRIOS (1997) classifies the genus of *Phytophthora* as a fungalike organism or pseudofungus. In this scheme, it belongs to the kingdom of chromista in the class of the oomycetes. This class is characterised by a non- separate elongated mycelium and the production of zoospores which are able to move by means of their flagella. Further, the oomycetes can develop durable, sexual resting oospores. The same author reports, that the species of *Phytophthora* cause a variety of diseases on many different plants, from vegetables and ornamentals to adult trees. Symptoms are as broad as the host range, reaching from the damping off of seedlings, over foliage blight to fruit rot. Most species however cause root and lower stem rots, that can provoke extremely destructive diseases in their hosts.

Morphology/ physiology

According to HARRACHI (2000) the mycelium consists of comparatively thick hyphae (6-8 µm) with the tips showing coralloid ramification. It usually grows in a rosette- like pattern. Under natural conditions, the production of sporangia is stimulated by soil bacteria (ZENTMYER, 1965) and represents the most important epidemiological step in the life cycle of *Phytophtora*, because in less than one hour 10 to 30 zoospores can be produced by each sporangium (ERWIN and RIBEIRO, 1996 in HARRACHI 2000). With twisting movements of their two flagella the zoospores mange to swim up to 6 cm in 10 hours through a sandy substrate in static water conditions (NEWHOOK et al., 1981, in CAMPBELL and BENSON, 1994). Hereby, they are attracted to host organs through chemotactic response across nutrient and ethanol gradients (ALLEN and NEWHOOK,1973 in CAMPBELL and BENSON, 1994). Once in reach of the host, infection is carried out by means of a germ tube. The mycelium grows inter- and intracellularly and invades cellular plasm via haustoria (SCHWERDTFEGER,1981; MOREIRA et al., 2006). The chlamydospores fulfil the function of securing the survival of *Phythophthora* under unfavourable conditions. They are ball-shaped and have thicker cell walls than the sporangia, which enables them to survive in the ground or in host- tissues for long periods of unsuitable conditions(MIRCETICH and ZENTMYER, 1966). Their germination can be stimulated by heightened nitrogen levels and root exudates of host plants (MIRCETICH et al. ,1968). For sexual reproduction gametangia- the oogonia and antheridia- are developed by meiosis (AGRIOS, 1997). Through a tube the contents of the antheridium are transferred into the oogonium. Then the oogonium swells and changes into a thick-walled oospore (HARRACHI, 2000). In this form it is able to survive up to six years (REUTER, 2005, internet 3) in infected roots or up to three meters deep in the soil (SHEARER and TIPPETT, 1989, in BRASIER, 1996).

Conditions required for reproduction

BRASIER (1992, 1996) emphasizes that *P. cinnamomi* is a soil- and water- borne pathogen that requires warm soils with a high water content or at least seasonal rainfall to enable the spread of the zoospores. His observation was that an attack is often especially severe in heavy soils, but can also be devastating in sandy substrates, seasonal rainfall provided. This corresponds with the suggestion of JUNG et al. (2000) that *Phytophthora* species are most widely spread on sandy- loamy, loamy, silty and clayey soils with a mean pH between 3.5 and 6.6. These findings are specified for *P. cinnamomi* by MOREIRA and MARTINS (2005), stating that the pathogen could be found in soils of all pH and textures examined, however, the highest rates were obtained from soils with fine textures and a pH above 5,1.

According to HARRACHI (2000), the minimum temperature for mycelium growth lies between 5 and 10° C, the ideal temperature between 24 and 28° C and the upper limit between 32 and 34° C. At very low temperatures (< 0° C) chlamydospores are even killed, at a level correlated with the degrees below freezing point (BENSON, 1982 in MAC DONALD, 1994).

The pathogen thrives well on moist, shady sites. Rainy springs and soils with a wet, compressed structure advance its development (SCHWEDTFEGER, 1981). Germination of the chlamydospores depends on temperature and moisture, while for the zoospores water serves as the transport medium (BENSON, 1994 in CAMPBELL and BENSON, 1994). The efficiency of the pathogen is increased linearly by prolonged periods of flooding (REYNOLDS et al., 1985). BRASIER et al. (1993) report that dying trees often occur in groups, with an affinity to stream sides, valleys or depressions. This corresponds with the observation of MOREIRA and MARTINS (2005) that the pathogen occurred more frequently on slopes and in valleys, than on hilltops.

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Distribution mechanisms

Under natural conditions animals may act as vectors, carrying infected soil particles with them (KLIEJUNAS and KO, 1976), but as mentioned above, water is the main factor in the classical spread of the zoospores. Hence the disease can only spread downhill or through lying surface water, without the aid of man. This fact should permit the tracing of the pathogen down to its source within an infested area.

Anthropogenic influence

ALI- SHITAYEH et al. (1991) proved that the zoospores can also be spread via irrigation water. As a logical consequence, wherever contamined water is transported, the pathogen is transported as well. In their survey on Hawaii, KLIEJUNAS and KO (1976) proved the presence of *P. cinnamomi* in mud samples adhering to tyres of vehicles and boots. BRASIER (1992) interconnects the occurrence of new rapid decline foci with recent soil perturbations (e.g. ploughing or road making) and changing land use patterns. The use of big machinery in forestry and especially intense ploughing underneath tree canopies, a technique called “limpeza” in Portuguese, might be a major contributory factor to the distribution of contamined soil particles. Chronologically the aggravation of the disease since the 1970ies fits in with the increasing mechanisation since this time. REUTER (2005, internet 3) points out the possibility of infection in nurseries and spread with planted seedlings.

Predisposing factors

Water stress was shown to increase the susceptibility to *P. cinnamomi* in the example of *Quercus rubra* (MARÇAIS et al, 1993). On the other hand, fine root losses due to a *Phytophthora* infection intensify the vulnerability to drought, as illustrated especially for *Quercus ilex*, which was the most susceptible of the five tree species surveyed by MAUREL et al. (2001). This leads to a vicious circle, culminating in the death of the tree (figure 5). Low oxygen concentrations in the soil also act as a predisposing factor on cork oak roots (JAKOBS,1991, in MAC DONALD, 1994). Generally, holm oak tissues were shown to be more susceptible to the pathogen than cork oak ones (MOREIRA- MARCELINO, 2001; PIRES et al., 2005 in MOREIRA et al., 2006). This further intensifies the threat for holm oak stands mentioned above.

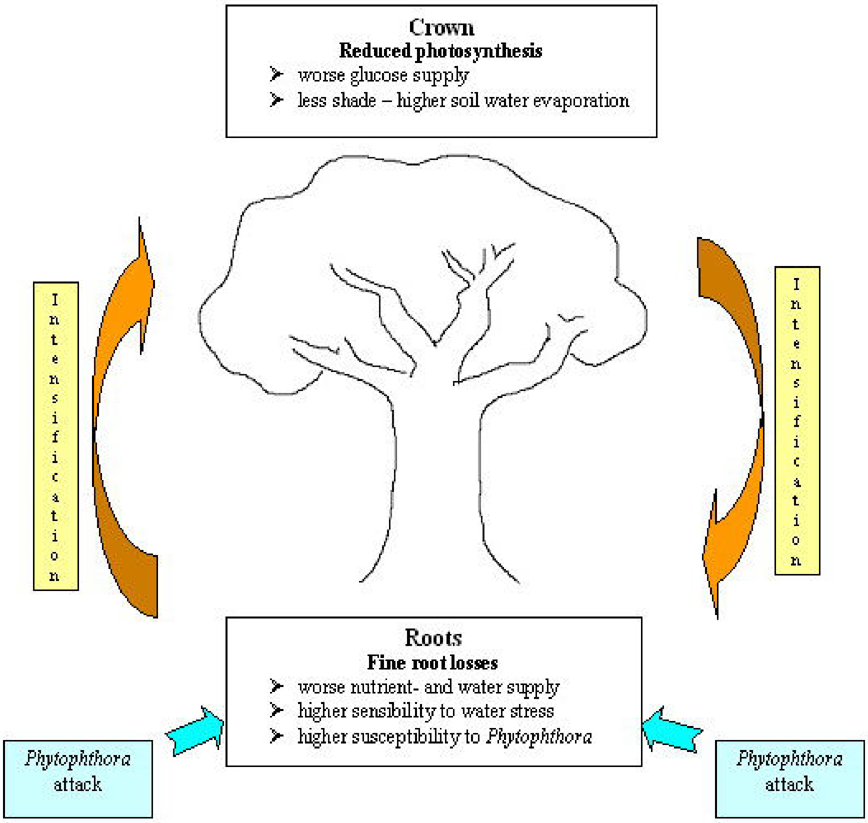

Fig. 5 Mutual reinforcement of *Phytophthora* infection and weakening of the tree

Brasier (1996) highlights that prolonged summer drought and heavy winter rain, as a consequence of climate warming, increase the susceptibility of the trees on the one side and the distribution speed of the pathogen on the other. On a computer model he simulates the intensification and further extension of the disease for the future. Meanwhile, another *Phytophthora* species - *P. ramorum* - which is known to cause sudden oak death in California (USA)- entered the Iberian peninsula (European and Mediterranean Plant Protection Organization, 2007, internet 4).This is all the more alarming as *Phytophthora* is known to be capable of bastardisation (Kehr, 2006, in Knebel, 2006). Another agent from the *Phytophthora* genus is *P. syringae*, which can cause fruit rot on citrus fruit (Capasso et al., 2001, internet 8) and a pruning wound canker on almond trees (Browne and Viveros, 1999). Further it has been isolated from sweet chestnut forests in France and Italy (Vettraino et al., 2001, internet 9). However this pathogen is considered rather harmless compared to other *Phytophthora* species (Browne and Viveros, 1999).

Pythium

The *Pythium* spp. are another genus from the class of the oomycetes. Like *Phytophthora* they are soil/ water- borne pathogens with a worldwide distribution. They can cause fruit rot on fruits and vegetables in contact with the soil and can infect seedlings and roots of all species. Young plants are the most endangered (AGRIOS, 1997). In forestry *Pythium* species are known to provoke the damping off of seedlings or to produce infected lesions on the root base of older seedlings that finally cause the plant to fall over (SCHWERDTFEGER, 1981).

2.3.2 *Biscogniauxia mediterranea* (former: *Hypoxylon mediterraneum*)

B. mediterranea is a globally distributed fungus best known for causing the charcoal disease in the Mediterranean region (SINCLAIR et al., 1987). The same authors consider it an opportunistic fungus, unable to cause damage on trees of normal vitality, however quick to colonise weakened, stressed or injured trees. In its sexual form, *B. mediterranea* belongs to the ascomycetes/ pyrenomycetes (HARRACHI 2000). The asexual form is described as *Botrytis sylvatica* Malençon (MALENÇON AND MARION, 1952 in HARRACHI 2000).

Chlorosis of leaves, crown dieback, bark cankers, tannic exudations, reduced ring growth and tree mortality are described as the main symptoms by VANNINI et al. (1996, b). These authors also report the occlusion of xylem vessels and a darkening of the surrounding parenchyma.

Fig. 6 Stromata of *B. mediterranea* on cork oak, inducing the cork layer to peel off.

Photograph by T. Kaltenbach, 6.III.2007

The most noticeable morphological attribute is the formation of stromata in the cambial zone of the tree (see fig.6). They are 1- 2mm thick, can reach dimensions ranging from a few centimetres up to several decimetres and are coloured black, with a roughened surface that resembles coal. The stromata develop raised edges that cause the overlying bark to crack and fall off (SINCLAIR et al., 1987; HARRACHI, 2000).Embedded in the stromata are large numbers of ball- shaped perithecia with a size of 0,5-1 mm x 0,4- 0,5 mm, that develop single celled asci, each of them containing eight ascospores. These ascospores show an ellipsoid shape and a brown colour in their ripened state, with a furrow from one end to the other (BREITENBACH and KRÄNZLIN, 1984). The asexual conidial form (*Botrytis sylvatica*) is only rarely encountered in nature. It appears as a powdered, light brown layer on top of the stroma (MALENÇON and MARION, 1952 , in HARRACHI 2000). In their survey of the factors affecting the discharge and germination of ascospores, VANNINI et al. (1996 a) determined precipitation or high relative air humidity as the main factors for the ejection of ascospores. They recorded that the ideal temperature for germination is 35° C,with temperatures between 25- 30° C as still favourable and 20° C and 40° C as reductive for germination. In the work mentioned above, VANNINI et al. (1996, a) found evidence that water stress is a main predisposing factor for infection. They highlight that periods of drought greatly increase the susceptibility to the fungus. Other diseases and pests weakening the tree can act as predisposing factors as well (SINCLAIR et al., 1987).CAPRETTI and BATTISTI (2007) showed at the example of *Lymantria dispar* that phyllophagous insect pests in combination with water stress raise the susceptibility to the pathogen. VANNINI et al. (1996, b) demonstrated ascospores to germinate better on wounded tissues than on non- wounded ones. This fact might be important especially in the case of *Quercus suber*, where through cork- cutting smaller or larger lesions regularly occur.

3 Material and methods

3.1 Description of the research area

The plot lies directly in the neighbourhood of the small village Corte Malhão which is situated in the Council of Odemira , in the Baixo Alentejo region, South- West Portugal.

Fig. 7 Location of the research area. Taken from Instituto Geográfico do Exército, 2006, internet 6

The annual average temperature, as measured in Beja, (247 m) is 16,2° C, medium precipitation/ year 585 mm (MÜHR, 2007, internet 7) and the climate shows a strong maritime influence.

The survey area is 2,27 ha in size and comprehends a small side valley which faces south-eastwards and ends in a larger valley facing south- west. The geological substrate is a clayey schist covered by a comparatively thin layer of clayey loam. This is valid especially for the northern hill, where the C- horizon (rocks) protrudes out of the soil. The boundaries of the research area were chosen in orientation to watersheds and waterlines as a limiting factor for the natural spread of *Phytophthora* (see maps I and II)

According to the landlord, the disease began about 15 years ago. The procedure of decline was described as very rapid in some cases, leading to death within several months (DA SILVA LUÇÃO, 26.II.2007, verbal communication). The territory is still used in the traditional way of the montado for the breeding of goats and pigs.

Fig. 8. View into the side valley **Photograph by T. Kaltenbach,13.III. 2007**

3.2 Survey of the research area and trees

The survey was carried out in two approaches, the first on February 8/ 9, 2007, the second on February 25/ 26 and March 2, 2007. The corner points of the plot, pathways , waterways and watersheds were recorded with their Gauss- Krueger coordinates by means of the portable GPS ‘Gecko 201’ by Garmin. The gradient of the area was measured with a Suunto gradient meter in percent. The coordinates of the trees were registered with the GPS mentioned above.

For each tree the following parameters were distinguished:

I tree species	V % of dead branches
II DBH (diameter at breast hight[1])	VI exudations
III damage class	VII year of last cork harvest
IV % of water sprouts	VIII additional remarks

Tab. 11 Parameters surveyed for each tree

Some of these parameters require further explanation:

II The DBH was measured with a diameter measurement tape. Only trees with a minimum DBH of 7 cm were registered. On trees branching into more than one trunk beyond DBH, the total diameter was determined with the derivation below:

$$\left(\frac{D1}{2}\right)^2 \times \pi + \left(\frac{D2}{2}\right)^2 \times \pi = \mathrm{A12} \Longrightarrow \sqrt{\left(\frac{A12}{\pi}\right)} \times 2 = \mathrm{D}_{\mathrm{total}}$$

The recorded measurements were assigned to DBH classes of 10 cm width, with class 0 reaching from 7 to 10 cm, class 1 reaching from 10 to 20 cm and so forth.

III The damage class was determined according to the instruction manual for the national forest inventory- IFN (DIRECÇÃO- GERAL DAS FLORESTAS, 1999), which uses the defoliation level as indicator (example photographs see annex 2). The IFN uses a 5- class system for the description of damages, which is depicted in table 2. It differs from the German level I programme by the introduction of damage class (Dc) 4 especially for

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[1] The breast hight is standardized to a hight of 1,30 metres above ground level.

declining cork oaks, which- independently of their age- do not offer conditions for cork extraction any more.

Damage class	Description	
Dc 0	No damage	0- 10 % of crown damaged
Dc 1	Light damage	11- 25 % of crown damaged
Dc 2	Moderate damage	26- 60 % of crown damaged
Dc 3	Accentuated damage	61- 90 % of crown damaged
Dc 4	Decrepit tree	Advanced state of decline, not permitting cork extraction any more
Dc 5	Dead tree	

Tab. 12 Damage classes according to the Portugese Nationl Forest Inventory (IFN)

However, this system turned out to be too inaccurate for the purpose intended here- especially in the second damage class, ranging from 26 to 60 % of damage. Therefore this class was modified. The main class 2, with an average at 42,5 % of damage was maintained in order to keep the data compatible with those of the regular forest inventories. To specify the state of damage within class 2, an extra group was formed, in which trees of class average were designated with the additional figure 2. Trees in an obviously better condition than class average were marked with a with an additional 1 and those evidently worse with a 3 (see table 3).

Damage class 2.1	better than class average	26- 35 % damage
Damage class 2.2	class average	36- 50 % damage
Damage class 2.3	worse than class average	51- 60 % damage

Tab. 13 Scheme for the subdivision of damage class 2

This system leaves the main emphasis (%) on the class average and emphasises only those trees evidently diverging from it.

Similar modifications were necessary for damage class 5 - the dead trees- in order to determine an infestation pattern for the whole plot and to try to discover the sources of the disease. For this cause, the dead trees were subdivided into four stages of decay:

Stage 1: twigs and leaves still left on the tree
Stage 2: twigs still left on the tree
Stage 3: branches of second order left on the tree
Stage 4: only main branches remained on the tree

Tab. 14 Decay stages of the dead trees

IV and V Water sprouts (adventitious shoots) and dead branches were recorded in addition to the basic crown evaluation, because in some cases leaves were just thinned out, without a significant percentage of dead branches occurring. Whereas, in other cases, the crown as a whole looked green at first glance, but with closer examination showed many water sprouts, a possible indicator for root stress (BRASIER, 1996). Percentage shares were estimated in 10 % steps.

VI Exudations were inspected in regard to the attributes localisation, infestation pattern, shape and depth each of them subdivided into the following categories:

Localisation	Infestation pattern	Shape	Depth
1 root base/ lower trunk	1 single	1 point	1 cambium bright/ not infected
2 main trunk	2 several	2 spot	2 cambium brownish/ interim state
3 upper trunk/ lower branches	3 massive	3 tongue shaped	3 cambium dark brown or black/ infected or dead
4 root base + main trunk		4 point + spot	
		5 point + tongue	

Tab. 15 Attributes recorded for the exudations

The depth of the necrosis was surveyed with a tissue drill with an inner diameter of five millimetres.

VII For *Quercus suber* the year of the latest cork harvest was recorded. The last number of the year is written onto the regrowing cork by the cork cutters. The 'virgin' trees, not harvested ever, were noted with a 0.

VIII The rubric additional remarks includes further observations, such as wind damage and infestation by phyllophagous insects. The latter were determined according to EBNER and SCHERER (2001); PATOČKA et al. (1999) and BOGENSCHÜTZ (1978). Additionally in this row the extraction points of the samples were noted in respect to the trees.

The evaluation of the data was implemented in Microsoft Exel and the map of the plot was created with ArcView GIS (version 3.1).

Topographical aspects

As *Phytophthora* is spread via water, the infestation of trees was evaluated in relation to their situation to the waterlines (see map II). These waterlines are ditches washed into the clayey substrate and bear water only in winter. Only those sections situated beneath decline foci were surveyed. This excludes the upper part of the main waterline. The southern waterline is distinctly smaller, than the northern one. Still it was included into the survey, because an evident attachment of single declining trees to its course could be noticed in the field. All trees within a 10 meter range of these waterlines were evaluated by their damage classes.

3.3 Survey in the laboratory

Because of the unreasonably high expenditure, the examination of samples was not carried out for each single tree, but just for 10 highly diseased sample trees. The main intention was to find evidence if/ which pathogens are present in the plot. As for *P.cinnamomi* MOREIRA and MARTINS (2005) regard a site as positive if at least one positive sample (root or soil) could be obtained from it.

3.3.1 Soil samples

The samples were gained from the research area on January 12, 2007, before the actual start of the tree survey. Soil samples were gathered from underneath eight heavily diseased trees. At each tree samples were taken at four points within a 1 metre circle around the tree, preferably under declining branches. They were extracted from a depth between 5 and 10 cm, mixed together and stored in a numbered plastic bag. The location of all extraction points was noted on a field map and the trees were marked with tape. Then the samples were stored at + 2° C.

The examination was carried out in the laboratory of the technical college Göttingen (HAWK) and guided by Prof. Dr. Kehr. On January 23, 2007, the soil-samples were put into Petri- dishes- four scoops for each dish- and filled up with water to 3/4 of soil level. All samples were stored under diffuse daylight at +20° C. On January 25, 2007, the samples were inspected for mycelium with an electronic microscope, magnified 25 times. Positive samples were magnified 200 times and inspected for non- septed mycelium characteristic for oomycetes. Photographs were taken for documentation and positive mycelia were transferred to separate 2 % water agar dishes with carrot pieces. Then the samples were stored as before for further observation and checked every two days. On January 29, 2007, young leaves of *Q. suber* were added to the soil samples as baits, 2 leafs per Petri- dish, floating above the sample. For a more detailed determination of the oomycetes, on January 29, 2007 additional shares of the original soil samples were sent to Dr. J. Schumacher at the National Biological Institute (BBA), Braunschweig. As the measurement of the pH value with Merck indicator sticks turned out to be too inaccurate, the values determined by the BBA for the 8 soil samples were taken as a base for the calculation of the medium pH value.

3.3.2 Tissue samples

Tissue samples were taken on January 12, 2007, from two infected trees showing increased exudation signs in the lower trunk regions. The cork was removed with a bark plane. Then the samples were drawn from the part around the necrosis in the transition zone between dead and healthy tissue and stored in separate, numbered plastic bags at +2° C. On January 23, 2007, the tissue samples were cut into 10 square pieces, 4 by 4 mm, then put onto a Heraeus ‘Lamin Air’ clean bench and disinfected in 96 % alcohol for 10 seconds. The pieces were then transferred onto sterile 2 % water agar with carrot chippings. Two pieces were placed in one Petri- dish. The dishes were stored under diffuse daylight at +20° C and observed every two days.

On March 2, 2007 a dead branch with a characteristic charcoal like infestation pattern was collected in the research area and brought into the laboratory on March 6, 2007. With a flamed scalpel, bits were scraped off and put onto a slide with a drop of distilled water. The slide was examined under 200- fold magnification and photographs were taken for documentation.

4 Results

4.1 Description of the stand and symptoms

The main tree species is *Quercus suber*, represented in all natural age stages from seedlings up to DBH 69 cm. The trees emerged from natural regeneration with different growth rates due to the localisation in the plot and disease intensity. Trunks are medium – short as favourable for cork extraction. The crowns are characterised by a prevailingly wide shape. Crown dieback could be observed all over the stand,particularly on the hill tops and valley bottom. Patches of wind damage and phyllophagous insect attack were noticed. Marginal shares of *Quercus rotundifolia* are associated in scattered mixture. The trees are distributed in a dispersed pattern, with gaps on hill tops and a more close attachment in depressions. Symptoms, as noticed at first glance, are similar to those described above for *P. cinnamomi*. The trees showed increased crown transparency, a notable percentage of dead branches and water sprouts. On single trees exudations or a chlorosis of the leaves could be observed (see figures 9 and 10). The most striking symptom, however, was the occurrence of distinct patch dieback, in some places including shrubs like *Cistus landanifer* and *Arbutus unedo*.

4.2 Results of the tree survey

In total 250 trees were recorded, with an average DBH of 29,71 cm. This corresponds to a population density of 110 trees/ ha. According to the best practice manual (DGRF, 2006 b), the ideal population density for this diameter amounts to 155 trees/ ha. Hence the population density index is 0,71. 243 of all the trees surveyed were *Quercus suber* and 7 (0,03%) *Q. rotundifolia*. This partial group is too small to allow representative conclusions for *Q. rotundifolia* and the values received did not vary significantly from those obtained for *Q.suber* (see table 6). The holm oaks are therefore not treated as a separate group throughout the further analysis. However, it may be noted that the holm oaks on this plot showed a slightly increased percentage of dead branches, compared to the cork oaks. Besides, the amount of water sprouts produced was a little bit lower than on the cork oaks.

	Average DBH total in cm	Average damage class	Average water sprouts in %	Average dead branches in %
Q. rotundifolia	29,51	2,60	14,97	37,00
Q. suber	29,71	2,50	15,74	34,06

Tab. 16 Selected values of *Q.rotundifolia* and *Q. suber* in comparison.

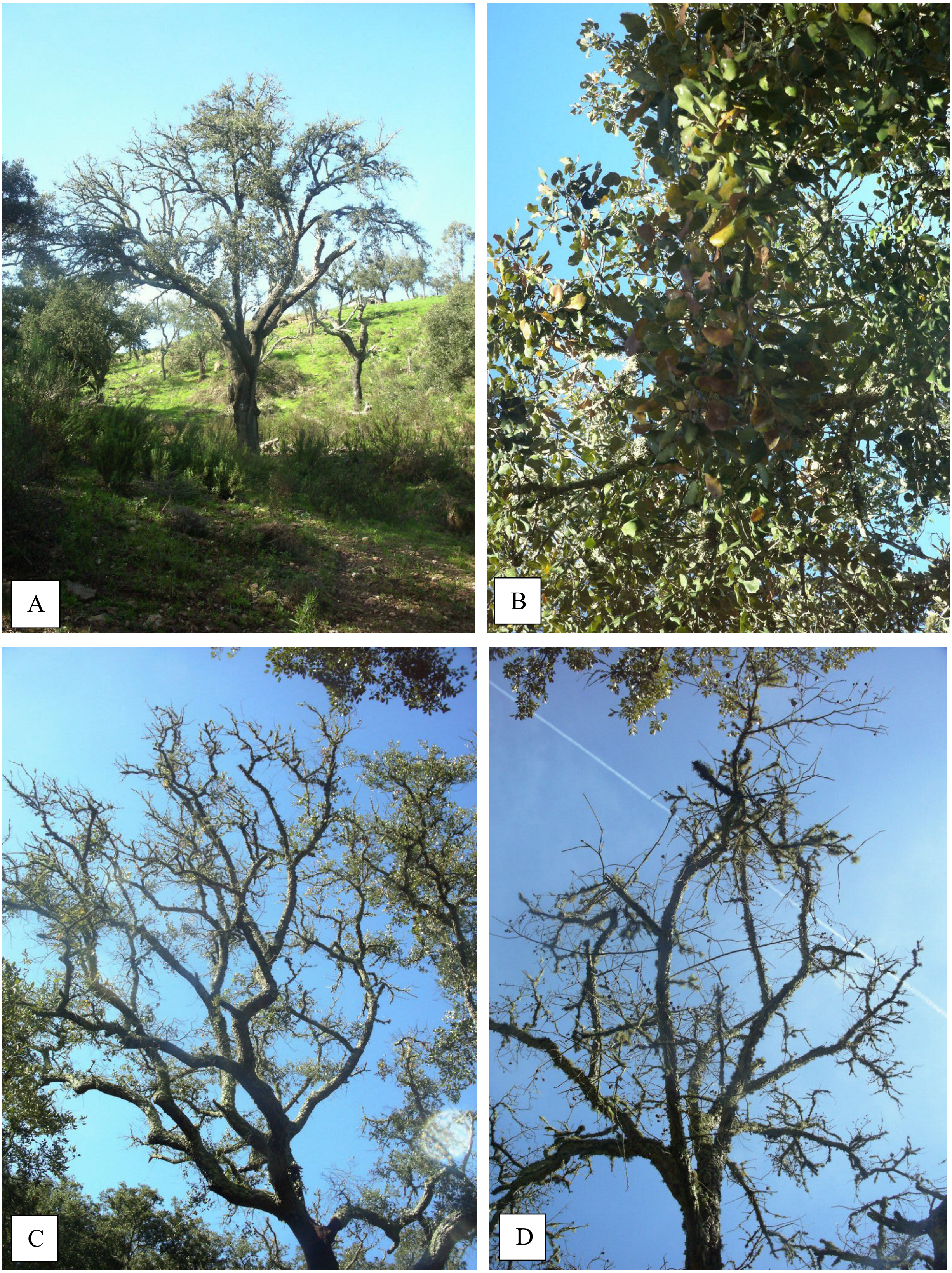

Fig. 9 Characteristic crown symptoms of *P. cinnamomi* observed on the plot

A Cork oak with a highly degraded crown (Dc 4) and a high share of dead branches
B Chlorosis and death of leaves
C Production of water sprouts (epicormic shoots)
D Remains of emergency fructification on a dead cork oak Photographs by T. Kaltenbach, 26. III. 2007

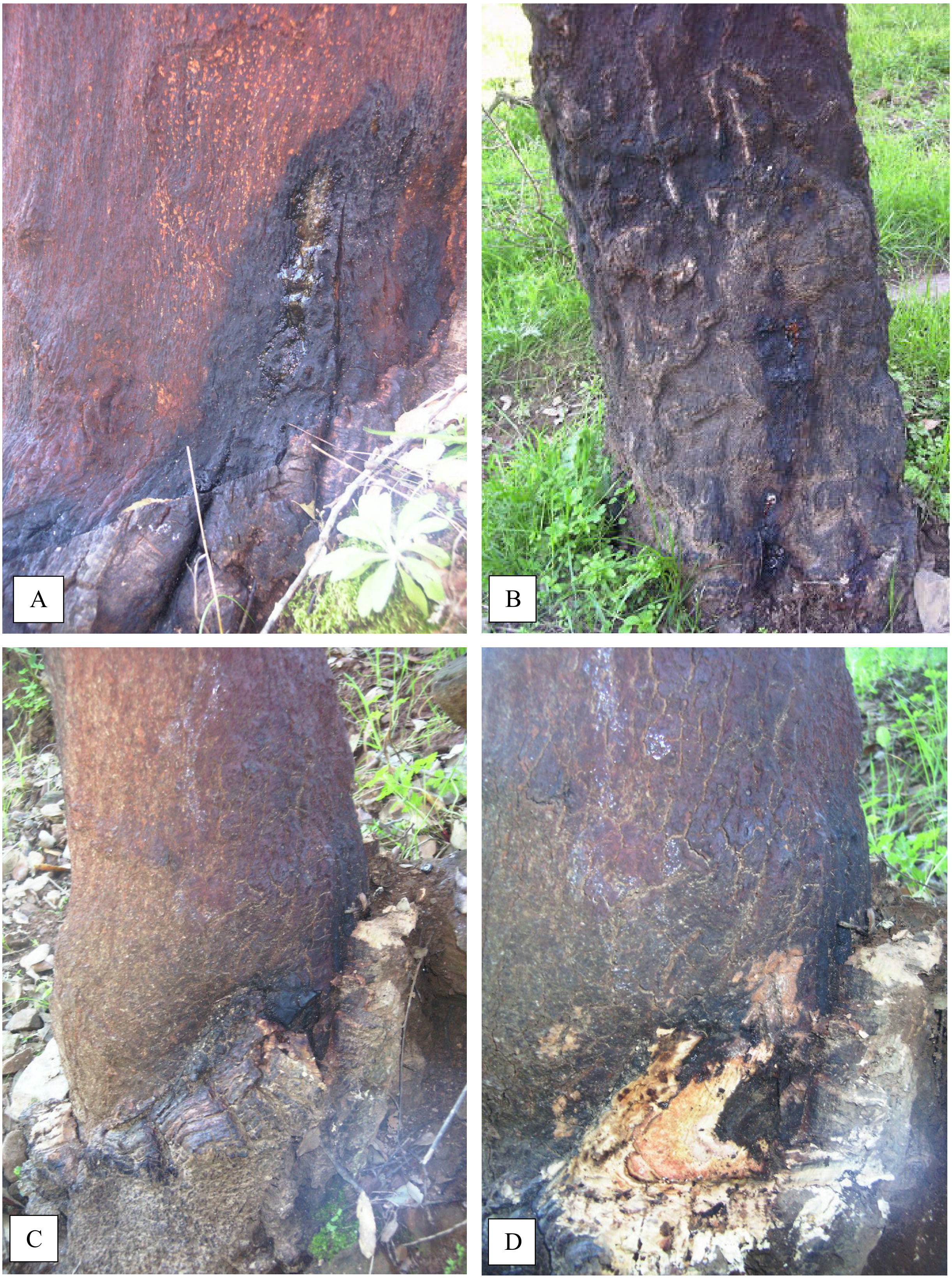

Fig. 10 Characteristic symptoms of *P.cinnamomi* at the root base/ lower trunk

A Tongue shaped exudation mark at the root base
B Tongue shaped exudation mark at the root base/ lower trunk
C Spot shaped exudation source at root base on infested cork oak
D Necrosis laid open with a bark plane on the same tree Photographs by T. Kaltenbach, 26.III. 2007

4.2.1 DBH and damage class distribution

DBH distribution

Trees of all natural age stages could be found in the research area, ranging from seedlings to strong trees with a maximum DBH of 90 cm (*Q. rotundifolia*). However, for a reflection on the DBH distribution, class 0 has to be neglected, because only trees above 7 cm were recorded and consequently this class is not representative. Taking a look at figure 11, an emphasis can be noted in DBH class 2 (trees ranging from 20 to 29 cm). From this class upwards a continual decrease of individuals can be observed. From class 2 to 9 the DBH distribution resembles an apportionment as to be found in the curve of a Plenter- forest or growth models for natural stands (BURSCHEL and HUSS, 1997). From this point of view, the sharp decrease from class 2 towards class1 is even more striking, because an even higher number of individuals within class 1 would be expected in comparison to class 2.

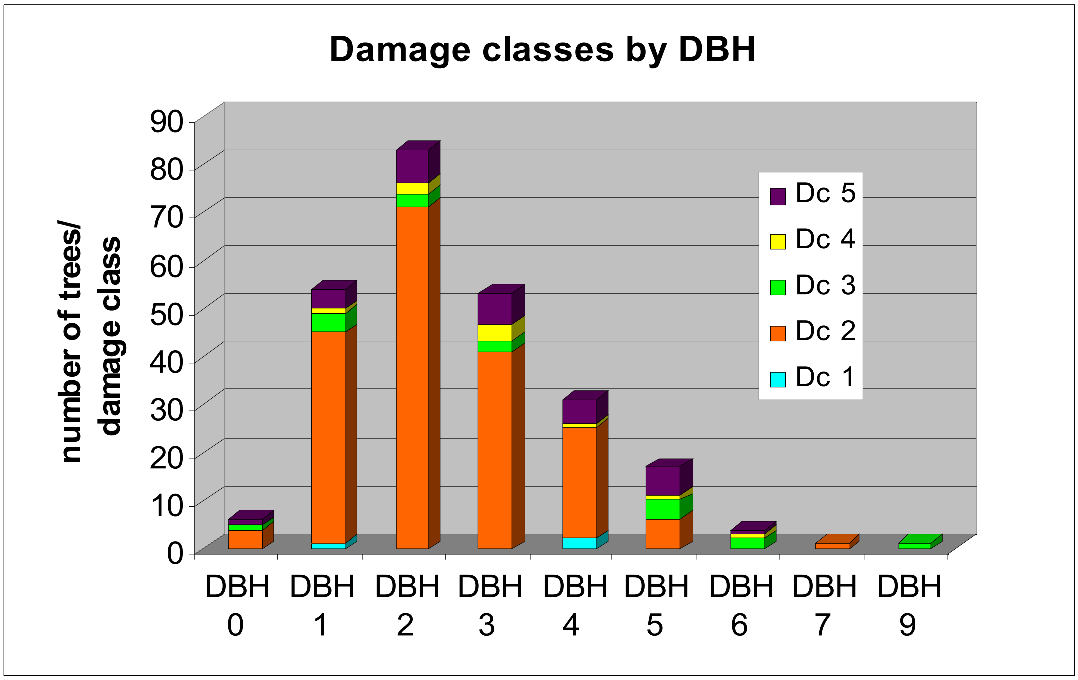

Fig. 11 Distribution of trees within damage classes (Dc) portrayed for the DBH classes

Damage class distribution

As can be seen in figure 12, damage class 2 , ‘moderate damage’, is by far the most widespread of all classes. Only 1,2 % (3 individuals) show light damage (Dc 1), while accentuated (Dc3) and heavy (Dc4) damages are represented with 6,8 and 4 %. A certain accumulation can be observed within damage class 5, the dead trees. On the one hand this may be due to the fact that trees that had died several years ago were also recorded in this class. On the other hand, several trees died as a consequence of a storm in the early winter. Closer examination of these individuals however showed that most of them were already weakened by wood decaying fungi before the weather event. Trees in a state of absolute health (Dc 0) were not found within the plot.

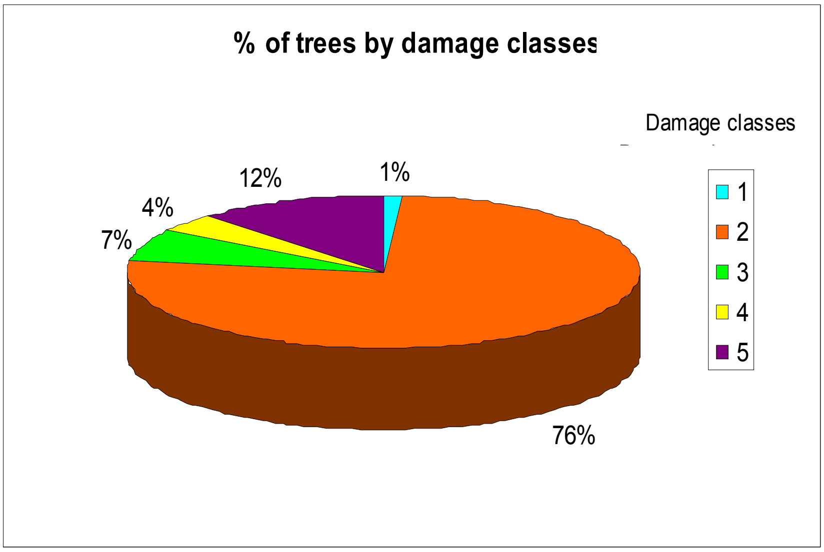

Fig. 12 Damage classes 1-5 and their % shares of all trees surveyed.

Relative shares of damage classes within the single DBH classes

Surveying the damage class (Dc) distribution within the single DBH classes (figure 13), larger shares of intensified damage can be noticed from DBH class 5 onwards (with the exception of DBH class7, which consists of only one individual). This trend culminates in DBH class 6, where no trees with moderate damage can be found any more.

Table 7 shows the mortality rate distribution for the respective DBH classes. The average mortality for the community of all trees is 12 %. Once again extreme values were recorded for DBH classes 5 and 6, each of them showing more than double the amount of the average mortality. Comparing these two classes, it can be deduced that although the total sanitary state is worse in DBH class 6, the highest mortality rate occurs in DBH class 5 with 35 % as opposed to 25 % in DBH class 6. Another important observation is that DBH class 0, the youngest trees surveyed, with 17 % of mortality are more seriously affected than the average of all trees.

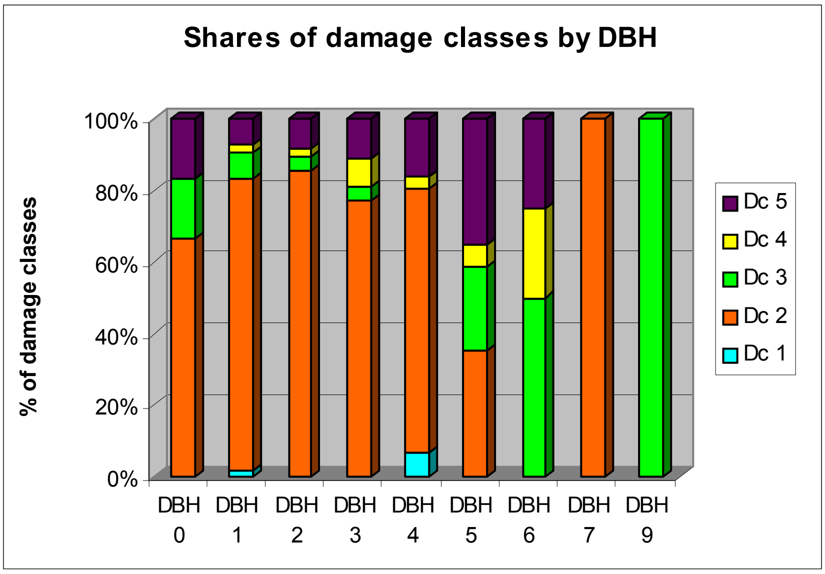

Fig. 13 Damage class (Dc) distribution within the single DBH classes in percentage shares

DBH class	% of dead trees (DC 5)
0	17 %
1	7 %
2	8%
3	11%
4	16 %
5	35 %
6	25 %
7	0 %
9	0 %
Average	12 %

Tab. 17 Shares of dead trees per DBH class

Closer observation of damage class 2

With 76 %, damage class 2 is the class containing the most individuals. But, as the matrix system for the Portuguese Forest Inventory is not built up linearly, it is also the class with the largest extensions in size (with 35 % of latitude). As a consequence in the field, considerable differences were noticed within the symptom range of the trees in this class. To gain a more detailed understanding of how this main class of the stand is structured, it was divided into three sub-classes, as described above, with the focus remaining in the class centre. The results can be observed in figure 14. 73 % of the trees remained in the medium range of the class

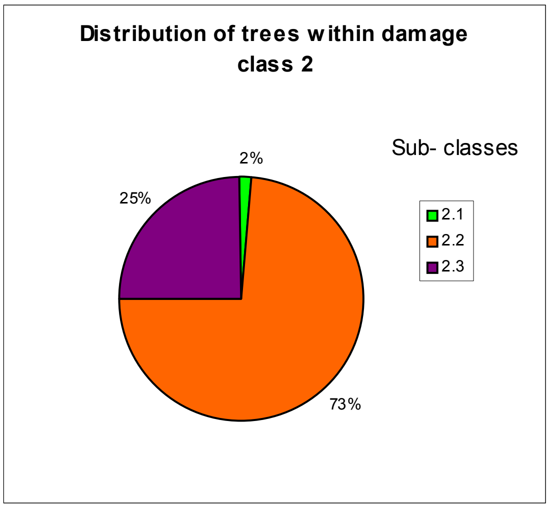

Fig. 14 Shares of the sub- classes within damage class 2

(2.2), whereas only 2 % were in an obviously better state (2.1). A quarter of the individuals however showed evidently worse symptoms (2.3). Hence within damage class 2 a trend towards the worse end of the distribution can be noticed.

Closer observation of damage class 5

The main intention of a more detailed survey of the dead trees was to get an idea of their distribution throughout the area. However, from the shares of the decay stages (1- 4) within Dc 5 certain developments can be derived as well. Figure 15 shows an accumulation of dead trees in decay stage 4, where only the trunk and main branches remained. This can be attributed to the process of natural degeneration. As a matter of fact, small twigs are catabolised more quickly than thick branches. Still decay stage 3, with the branches of 2nd order still left on the tree, is the most rare class and only two individuals were found throughout the whole plot. Contrary to this, decay stages 1 and 2 (dead leaves and twigs/ just twigs left on the tree) occurred distinctly more often, even if the four individuals, which had fallen a victim to storm (within class 1) were excluded. This allows the logical conclusions that

1 the disease does not proceed linearly, but rather in waves, and
2 at the present point in time a more offensive phase is taking place.

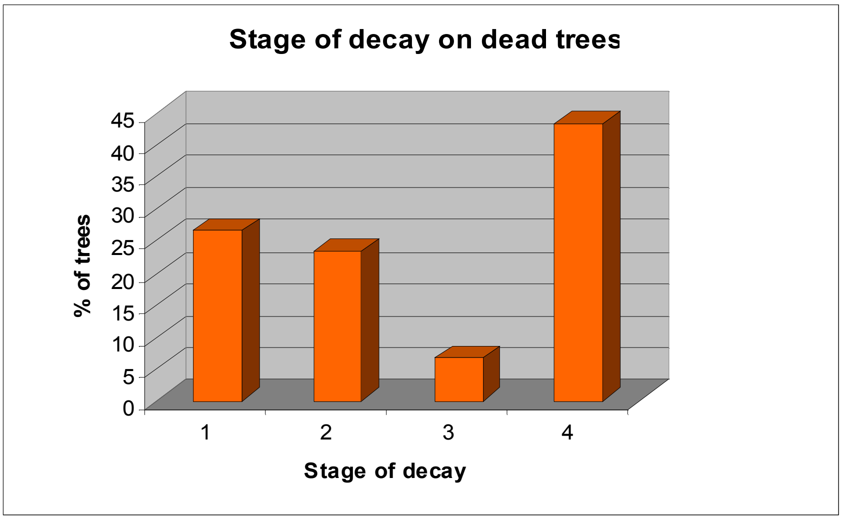

Fig. 15 Shares of trees in decay stages 1- 4, within damage class 5 (dead trees)

4.2.2 Crown symptoms- shares of water sprouts and dead branches

These two parameters were assessed in addition to the IFN guidelines, in order to find out about the vitality status of the trees. Figure 16 shows how both characteristics increase linearly with growing damage intensity represented by the damage classes. Correlation tests proved highly significant levels for both attributes with R^2= 0,9242 for water sprouts and R^2= 0,9842 for dead branches (see annex 11).

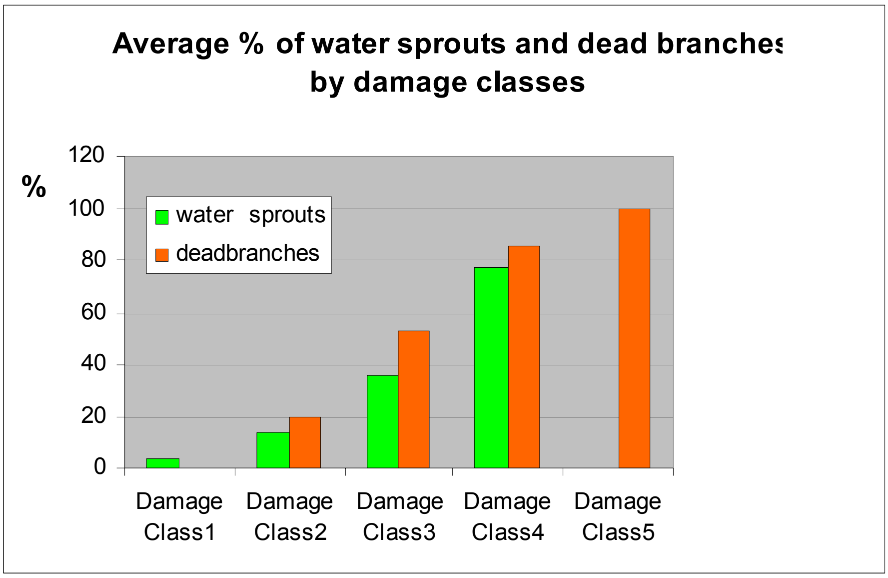

Fig. 16 Average shares of water sprouts and dead branches for the different damage classes in percentages.

Still, on the level of the single trees great differences from these class average values occurred. In figure 17 the standard deviation from the class average is portrayed for these two parameters. For the dead branches, the deviation culminates in Dc 3, with a maximum of 18,63 %.For the water sprouts however, the maximal values reach up to 34,11 and 35,61 % in the damage classes 3 and 4. This shows that trees in an advanced state of disease react very differently in the production of water sprouts. Further, it can be noted that the discrepancy between both values increases linearly with rising Dc level from Dc 2 to 4 (no standard deviation occurred in Dc 5 and for the dead branches in Dc 1). Significance was tested with of R^2 = 1,0 (see annex 12). This means that the differences in deviation of the two symptoms become more obvious with increasing intensity of the disease. For this reason, these two symptoms were submitted to closer examination.

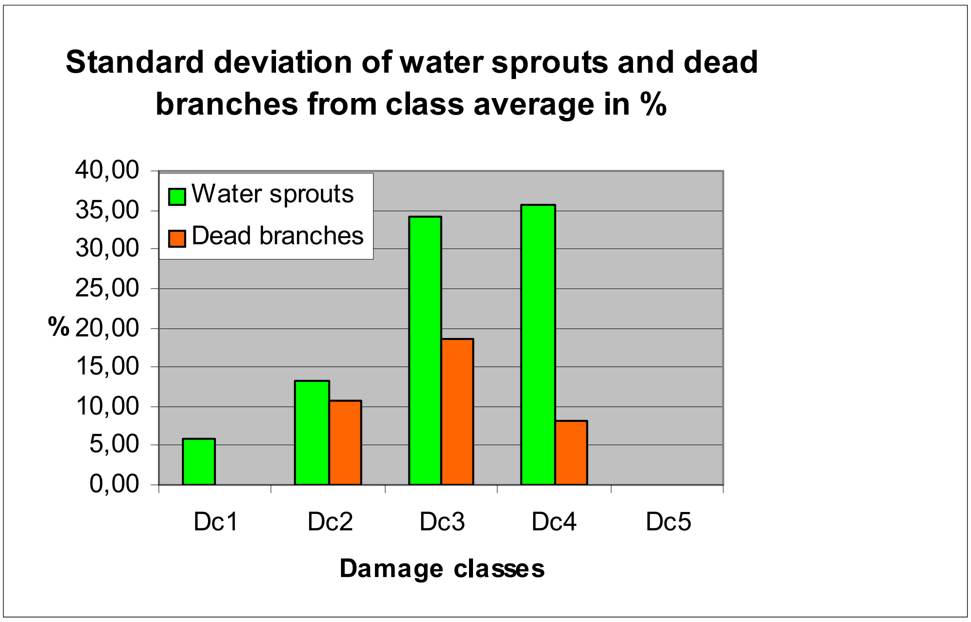

Fig. 17Standard deviation of water sprouts and dead branches within the different damage classes.

Shares of water sprouts and dead branches in relation to DBH classes

As to be observed in figure 18, the highest shares of dead branches occurred within DBH classes 5 and 6, which were also the classes with increased Dc levels (see fig. 13). An evident increase of water sprouts can be noticed from DBH classes 5 and especially 6 onwards, approaching dead branches level in DBH class 6 and even outgrowing it in DBH classes 7 and 9.

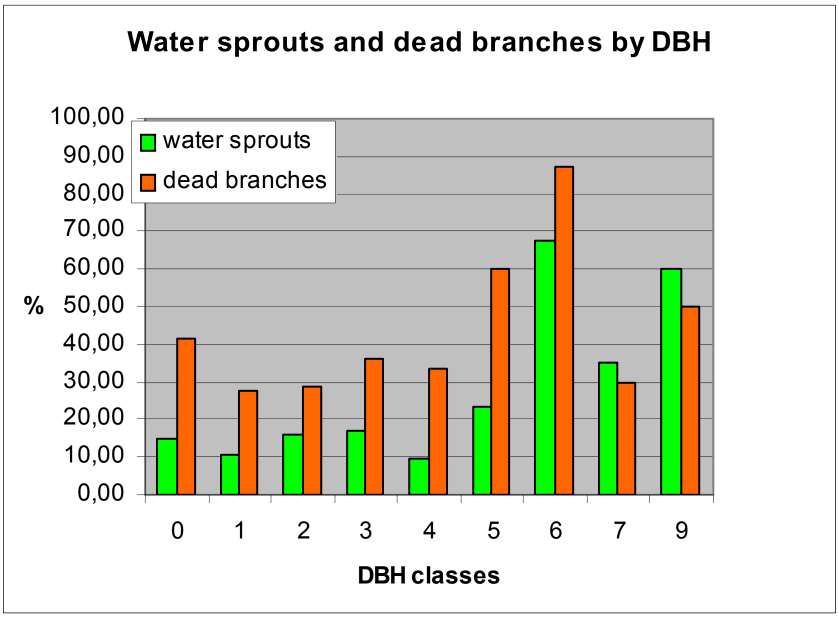

Fig. 18Correlation of water sprouts and dead branches to DBH classes

DBH class	index ws/db
0	0,36
1	0,39
2	0,55
3	0,47
4	0,28
5	0,39
6	0,77
7	1,17
9	1,20

Tab. 18 Index water sprouts/ dead branches

4.2.3 Exudations

For 16 of the dead trees exudations could not be distinguished any more. Of the remaining 234 individuals, 150 (64%) did show exudations. Table 9 shows the different parameters recorded for exudations in percentages. The dates recorded for the exudations at each tree can be seen in the fieldbook (annex 1)

Localisation

In the evaluation of exudations, the localisation was the first parameter recorded because it already gives valuable hints to possible causes. As described above, *P. cinnamomi* invades the tree through the roots, while *B. mediterranea* can use fine branches or wounds as an entry and consequently is more likely to be found in the upper compartments of the tree. Moreover, root base and bifurcation are the parts of the tree, the most endangered by cork extraction, because here the cuts are carried out crosswise to the vessels. However wounds caused by cork extraction without secondary infection were not considered in the evaluation. Out of all exudations, 73 % were situated at the main trunk, 17 % at the root base and 5 % on the upper trunk. Another 5 % of trees showed exudations on both trunk and root base (see table 9).

Infestation pattern

The ‘infestation pattern’ describes the frequency of exudations on the tree and characterises the intensity of the attack by quantity. Two thirds of the affected trees showed medium to strong infestation (see table 9).

Attributes of exudations	Manifestation of attributes in percentage shares					Total
Localisation	1 root base	4 root/trunk	2 trunk	3 upper trunk		
	17,33	4,66	73,33	4,66		99,98
Infestation pattern	1 single	2 several	3 massive			
	35,33	50,66	14			99,99
Shape	1 point	2 spot	4 point/spot	3 tongue	5 point/tongue	
	53,33	24	7,33	12	3,33	99,99
Depth	1 cambium bright	2 light brown	3 cambium dark			
	6	10	84			100

Tab. 9 Results of the exudation survey; manifestation of the attributes for each of the four parameters recorded.

Shape of the exudation sources

The parameter shape characterises both the shape and the size of the exudation marks. By closer examination it can be noted that

- on the main trunk 78 % of exudations were point and/or spot shaped (figure “Shape of exudations on main trunk”- annex 5). Spot-checks revealed that most of point and spot shaped exudations originated from insect boreholes or small wounds.
- At the root base 42 % of the exudations (36,4 % of all trees surveyed) showed tongue- or tongue and point shapes, a typical symptom of *Phytophthora* infection (figure “Shapes of exudation sources at root base”- annex 5).

Depth of the exudation sources

Another key parameter is the depth of infection sources. It provides information as to whether the cambial tissue is involved in the process or not. A dark brown or black colouration indicates a cambial necrosis. This was the case for an alarming 84 % of the trees with exudation marks.

Correlation between exudations and water sprouts

The 150 individuals (60 % of all trees) with exudations oppose to 146 (58 %) with a share of water sprouts above 10 %. Only 40 trees (16 %) did not show exudations nor water sprouts.

For both trees with and without exudations, the curves of water sprout shares proceed in a slightly undulating course with a clearly descending tendency (see fig. 19). Significance was tested, with $R^2 = 0,94$ for trees with exudations and $R^2 = 0,89$ for trees without exudations.

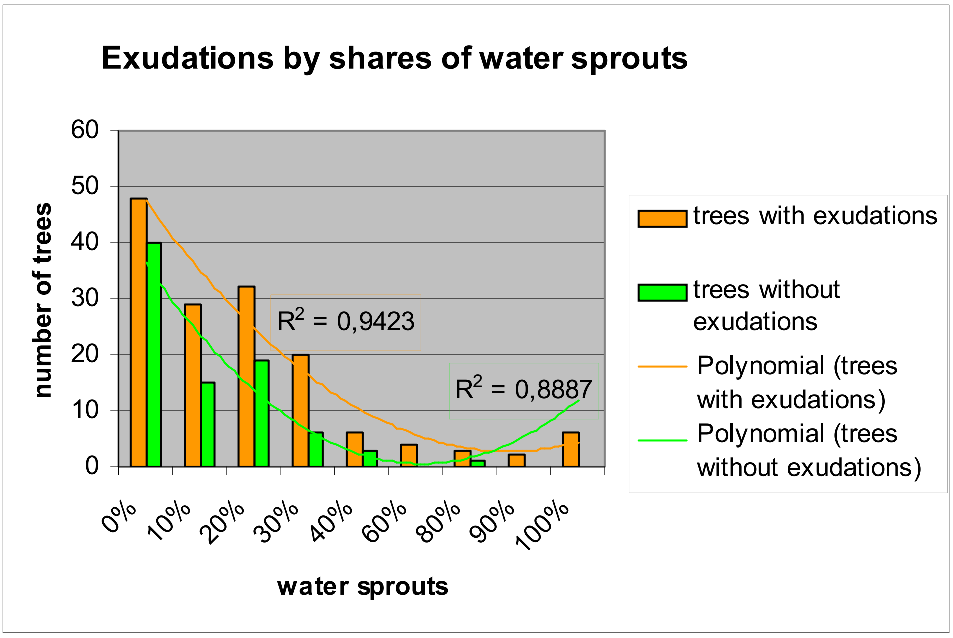

Fig. 19 Correlation between exudations and the percentage of water sprouts

From this distribution several things can be concluded:

1 Throughout the whole stand, trees with lesser water sprout shares occurred much more frequently than those with high water sprout manifestation.

2 Trees with exudations are more common than those without throughout the whole distribution.

3 From 60% of water sprouts onwards all trees showed exudations, with the exception of one individual (at 80%).

4 The relative shares of trees with exudations are positively correlated with the amount of water sprouts produced. Significance test revealed $R^2 = 0,73$ (annex 3a). On the contrary shares of trees without exudations are negatively correlated with a significance of $R^2 = 0,72$ (annex 3a).

The attributes of the exudation sources- localisation, infestation pattern, depth and shape- were related to water sprout formation, but no significant correlations could be calculated (see annex 3b- characteristic *Phytophthora* symptomatic marked in orange). Still one observation can be derived from the figure “Depth of exudation sources” (annex 3b): all trees with more than 60% of water sprouts showed a cambial necrosis, with the exception of the one individual mentioned above (with 80% of water sprouts).

Correlation between exudations and shares of dead branches

212 individuals (91 % of all trees) showed a share of dead branches above 10%. 17 trees (7%) did not show either of the both symptoms. Out of the 150 trees with exudations, five did not show a recognizable amount of dead branches. Figure 20 correlates the amount of dead branches with the occurrence of exudations. It can be recognized that:

1 the curves for the dead branches take a two peaked course with an absolute maximum of 78 individuals (31 % of all trees) between 20 and 30 % , then declining and rising again from 60 % towards the end.

2 From 20 % of dead branches onwards there are evidently more trees to be found with than without exudations.

3 From 50 % of dead branches onwards all trees do show exudations.

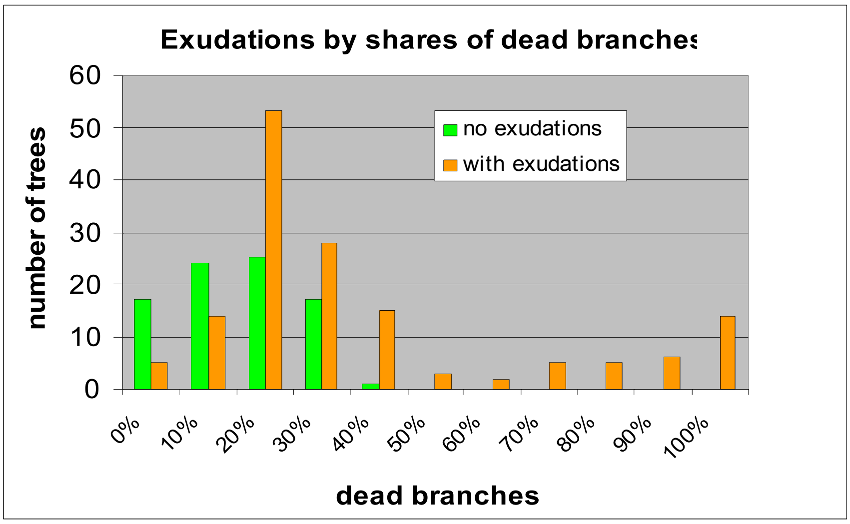

Fig. 20 Correlation between exudations and dead branches percentage

Taking a closer look at the nature of the exudations two assessments can be deduced:

1 A cambial necrosis can be determined for 90 % of the trees with a share of dead branches above 40% (figure “Depth of exudation sources” annex 4).

2 An enhanced occurrence of tongue shaped exudations can be noted from corresponding 80% of dead branches onwards (figure “Shape of exudation sources” annex 4).

Distribution of exudations within the damage classes

The distribution of trees with exudations within the damage classes is portrayed in figure 21. Comparing these shares with those of all trees (see figure 12), it occurs that

- no exudations were recorded in Dc 1.

- Damage classes 2 and 5 decreased in size by 3 % each, compared to the shares calculated for the respective classes for the community of all trees. This means that in Dc 2 exudations occurred less frequently. As exudations could not be distinguished for half of the individuals in Dc 5 any more, this group has to be regarded with care. An actually higher value can be assumed for this class.
- Damage classes 3 and 4 increased by 4 % and 3 % respectively.

From these observations it can be deduced that trees with exudations were mainly distributed similarly to the community of all trees throughout the damage classes. This again allows the conclusion that exudations can occur in all damage classes (apart from Dc 1) with almost the same probability, however, with a slightly higher incidence in an advanced state of damage.

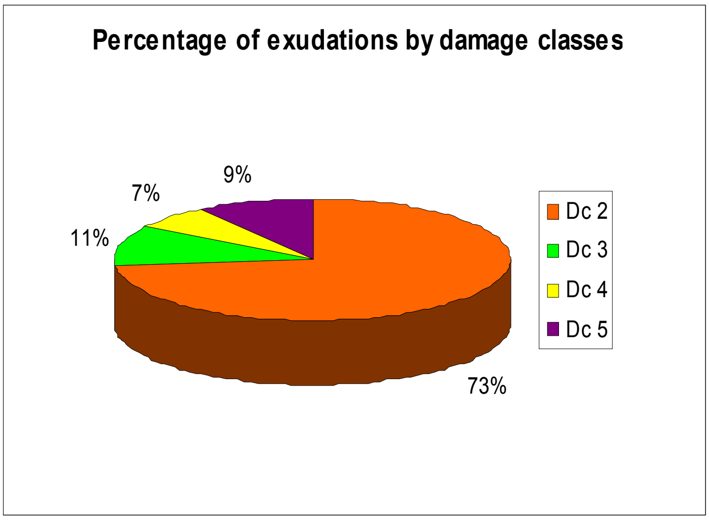

Fig. 21 Distribution of exudations within damage classes

Surveying the attributes of exudations throughout the whole stand it can be derived that, even though Dc 2 is by far the most widespread class, the depth of the exudation sources is drastically increasing from Dc 3 onwards. A cambial necrosis of 47 % in Dc 2 is rises to an average of 87 % in classes 3- 5 (figure “Depth of exudation sources” annex 6). Most blatant however, is that the curve for the tongue shaped exudations (shapes 3 + 5) shows an approximately polynomial growth rate throughout the whole distribution with a maximum of 57 % in Dc 5(figure “Shapes of exudation sources” annex 6). Significance was tested with $R^2 = 0,96$ (annex 7). The curve for the massively infested trees takes a similar course. Massively affected trees vary from 5 % in Dc 2 to 50 % in Dc 5 (figure “Infestation pattern of exudations” annex 6). Here the significance amounted to $R^2 = 0,90$ (annex 7).

Correlation between year of the latest cork harvest and the damage class

The cork extraction could be retraced over a period of 15 years- until 1992. In absolute numbers, the biggest shares are held by the 'virgin' trees and the trees harvested in 1997 with 30 % each. For 6 of the dead trees marked with a '?', the year could not be identified any more.

Figure 22 depicts the relative shares per damage class. It is remarkable that the trees harvested last (2006) are in a prevailingly good condition and only a single tree died as a consequence of the cork extraction. This is probably due to the fact that usually only trees in good health are harvested (DGRF, 2006 b). In both of the two other groups big enough to be called representative, the years 2002 and 1997, a drift can be observed, either to the better, or to the worse end of the distribution. The trend to the worse end however, is more pronounced for the group harvested further back in time- 1997. The most striking observation is that the 'virgin' trees constitute a considerably bad part in the distribution. To gain a more detailed understanding of this situation it is necessary to compare the shares of this special class represented in the respective damage classes (annex 8a) to the shares held by the community of all trees (see figure 12). In Dc 2, the main class, 76% for all trees are found as opposed to only 74% for the virgin trees. Damage classes 4 + 5 are represented by 16 % of either group. The biggest gap however occurs in Dc 3, where 4 % for all trees are topped by 10 % for the virgin trees.

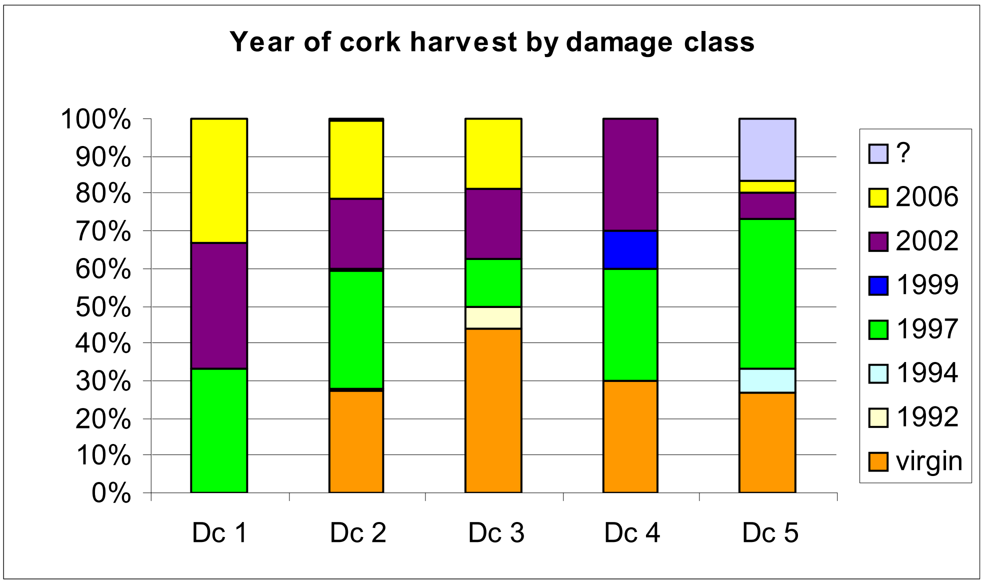

Fig. 22 Correlation between year of latest cork harvest and damage class

4.2.4 Topographical aspects

The medium gradient of the research area, as an average of five measurements, was calculated with 37 %. This means that water flows off quickly in most part of the plot. As a result very dry conditions can be observed on the upper slopes, and moist conditions in the valleys. Further this means good conditions for the spread of *P. cinnamomi*. To survey this paticular task,the trees were examined in regard to their position to the waterlines, where a concentration of this pathogen can be assumed. The result is presented in figure 23. Compared to the shares for the community of all trees (figure 12) a decrease in the classes of lower damage is opposed by an increase in the higher damaged classes. No individuals out of Dc 1 were to be found in a 10 metres range of the watersheds. For Dc 2 a minus of 7 % was recorded compared to the share of this damage class for the whole stand. An increase of 1% in Dc 3 is raised to 3% in Dc 4 and 4 % in Dc 5 (see annex 10). This trend is portrayed in figure 24. A polynomial increase of the damage classes near the waterlines could be detected. The significance test revealed a level of $R^2 = 0,98$.

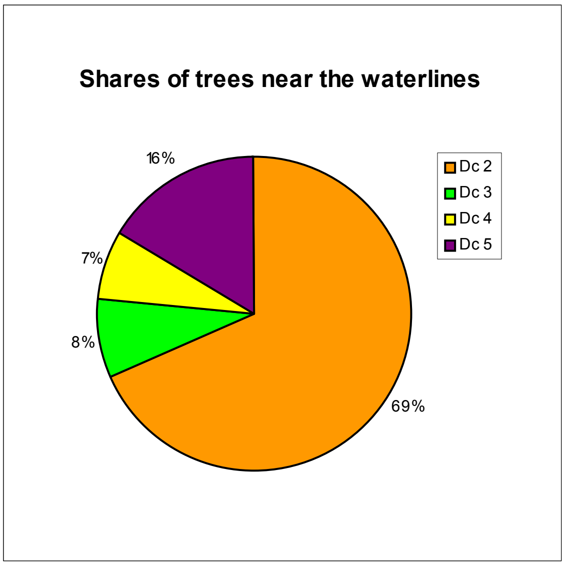

Fig. 23 Shares of trees per damage class within a 10 meter distance of the waterlines

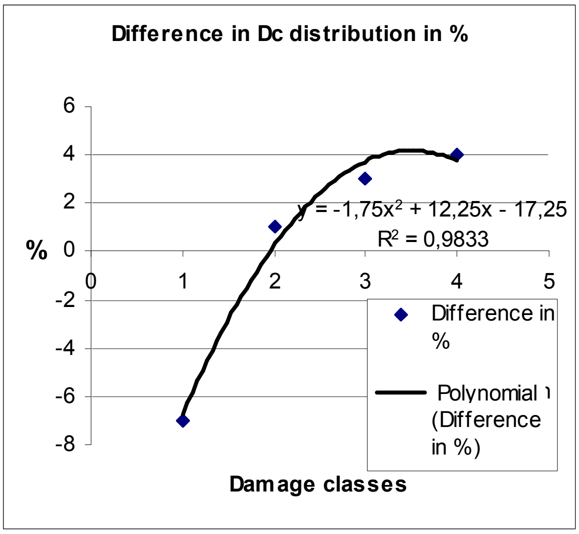

Fig. 24 Differences in damage class distribution between the community of all trees and trees near the waterlines

Still only 46 % of the dead trees are situated near the waterlines and indeed another decline focus could be found on the south east facing northern slope (see map II). For 3 out of 12 dead or dying trees located in this region the occurrence of “charcoal coloured patches under peeling bark" was explicitly noted in the column "remarks" (annex 9).Further the sample surveyed for *B. mediterranea* was collected in this region. Hence in this area an intensification of the decline can be ascribed to *B. mediterranea*.

This region is also the area, where according to the landlord the first signs of the disease were noticed about 15 years ago (DA SILVA LUÇÃO, 26.II.2007, verbal communication). This statement can be confirmed, on the example of tree number 112. It was harvested for the last time in 1997 and died soon afterwards (no new cork layer was built up). Two trees in its surrounding must have died even further back in time, because here the year of the last harvest could not even be distinguished any more. This indicates that the disease must have started more than 10 years ago.

Deviations of the coordinates received from the GPS have to be noticed. They are probably due to a radio interference by the surrounding hills. The coordinates of 7 trees were registered out of the limits of the research area. The strongest interference occurred in the narrow part of the upper side valley (see map II). Still the map as a whole can be called representative.

4.2.5 Additional Remarks

Wind damage occurred on 0,5 % of the trees due to a storm in the early winter. The presence of phyllophagous insects such as *Rhynchaeus quercus, Phyllobius argentatus* and caterpillars of *Tortrix viridana* was determined in patches (on 1 % of the trees). On the first visit to the area on February 8/ 9, 2007, no insects were noticed yet, while on the second approach on February 25/ 26 and March 2, 2007, a presence could be noticed. As the survey was carried out in early spring, however, the damage was still considerably small and negligible for the assessment of the crown conditions.

4.3 Laboratory Results

4.3.1 Soil samples

The mean pH value determined out of the 8 samples tested by the BBA (see annex 8 b) revealed 5,60. This means very favourable conditions for the growth of *Phytophthora*.

The inspection of the soil samples for oomycete mycelium delivered the results portrayed in table 10. Four out of the eight samples were found to be positive for oomycetes after two days. After ten days however the oomycetes in the original petri dishes, as well as, the isolates taken from them were overgrown by other fungi, as no selective mediums were used. Leaf baits were colonised by leaf decomposing fungi very quickly.

Sample 1	negative for oomycetes
Sample 2	negative for oomycetes
Sample 3	positive for oomycetes
Sample 4	positive for oomycetes
Sample 5	negative for oomycetes
Sample 6	positive for oomycetes
Sample 7	positive for oomycetes
Sample 8	negative for oomycetes

Tab. 10 Results of microscopic survey of the soil samples after 2 days.

Fig. 25 Non- septed oomycete mycelium under 200 fold magnification.

Photograph by T. Kaltenbach, 25. I. 2007

Table 11 shows the results of the examinations carried out by the BBA (BIOLOGISCHE BUNDESANSTALT, 2007). Out of the eight samples surveyed five were positive for *P. cinnamomi*. These samples contained very high quantities of the pathogen, which implies a high pathogenity for *P. cinnamomi*. Moreover, 2 samples revealed contamination with *P. syringae*, and *Pythium* spp. were discovered in five of the samples. Summarising, it can be stated that all of the samples did contain soil borne pathogens. As these pathogens are not easily isolated (HARRACHI, 2000), a high degree of contamination by these oomycetes can be assumed for the whole plot.

	sample 1	sample 2	sample 3	sample 4	sample 5	sample 6	sample 7	sample 8
P. cinnamomi	+	+	-	+	-	+	+	-
P. syringae	+	-	-	-	-	-	-	+
Pythium spp.	+	-	+	+	+	+	-	-

Tab. 20 Results of the BBA survey of the soil samples

4.3.2 Tissue samples

No evidence was found for oomycetes on the tissue samples. The regions around the cambial pieces were visibly colonised by bacteria after one week. However, on the branch collected on March 2, 2007, stromata and ascospores of *B. mediterranea* could be determined (see figure 26).

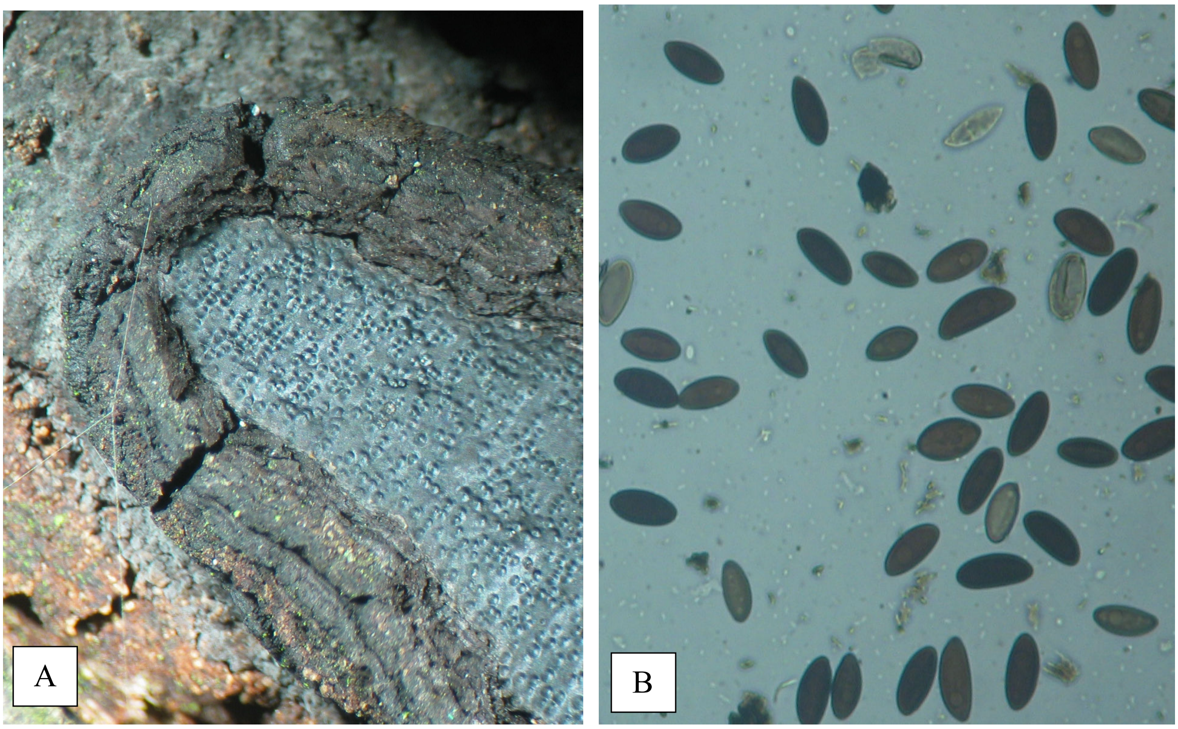

Fig. 26 Stromata and ascospores of *B. mediterranea*

A Stromata under 25 fold magnification
B Ascospores under 200 fold magnification, photographs by T. Kaltenbach, 6. III. 2007

5 Discussion

Distribution of damage

Of the 250 trees recorded, all showed at least light damage (3%), the majority however moderate damage (76 %). Accentuate damage was noticed on 7 % of the trees and 16 % were either dead (12 %) or dying (4 %). This number still appears relatively small compared with the description of MONTOYA (in BRASIER, 1996) and BRASIER (1996), who stated that half of the trees in a typical decline focus can be considered dead or dying. The definition given by the Portuguese authority in annex I b of the PDPS considers an area a decline focus, if 25 % of the trees are dead or decrepit (MINISTRO DA AGRICULTURA, DESENVOLVIMENTO RURAL E PESCAS, 2003). Even though the actual state of the surveyed area is already devastating, these descriptions allow the conclusion that things could well get even worse. This apprehension is confirmed by the recognition gained from the survey of the dead trees on the plot, yielding that the disease has been proceeding in waves and that currently a rather offensive phase is taking place. This phenomenon can probably be ascribed to the interaction of the disease with climatic factors, such as winter droughts, leading to water stress on the trees (BRASIER et al., 1993).

Involved pathogens

The examinations of the soil samples in the laboratory revealed a high contamination with oomycetes, especially *P. cinnamomi,* which is a highly aggressive primary root pathogen. For this agent, five out of eight samples were positive. This is a comparatively high rate, taking into consideration that the isolation from the soil is considered difficult because of the low population density of the pathogen in the soil (ERWIN and RIBEIRO 1996 in HARRACHI 2000). BRASIER (1992) proposes that the problems in the isolation from the soil are due to low soil water contents. This suggestion can be confirmed, as 4 of the 5 positive samples came from the valley bottom, where moist soil conditions did exist. In contrast, stromata of *B. mediterranea* were discovered with an affinity to the uphill sites with a southern exposure. This fits with the suggestion of VANNINI et al. (1996, a), who showed water stress to be the main predisposing factor for this opportunistic parasite.

Considerations concerning the age distribution

In regard to the DBH/ age distribution, the assessments revealed that young trees (DBH class 1) are under- represented in the stand. This might be due to several reasons. Changing land use patterns and increasing soil disturbances (BRASIER, 1992; BRASIER et al.,1993) were accompanied by the successive introduction of heavy ploughing machinery into the region since the 1970ies (DA SILVA LUÇÃO, verbal communication, 26.II. 2007). On the one hand ploughing underneath the tree canopies in itself is certainly detrimental for natural regeneration. On the other hand these machines may also act as a transport vehicle for *Phytophthora* (KLIEJUNAS and KO, 1976), which can kill seedlings much more easily than adult trees because of the lesser development of their root system. The *Pythium* spp as well might have contributed to the damping off of seedlings (AGRIOS, 1997). Another contributory factor in this context might be the goat breeding, which can cause severe damage in the understorey (DGRF, 2006 b). The breeding of goats and pigs on the land may also have promoted the further spread of the pathogen within the plot (KLIEJUNAS and KO, 1976).

The next observation made, in the context of age distribution, is that the 'virgin' trees are slightly higher affected by damage than the average of all trees. It is important to know in this context that 65 % of this group come from DBH classes 0 + 1, 29 % from DBH class 2 and only 6 % from classes 3 + 4, which means that this class includes mainly young trees. Another consideration is that a cork- extraction always weakens the tree (DGRF, 2006 b), so that actually a better state of the virgin trees could be expected. The reason why the opposite case was observed, is probably due to the fact that the cork- cutters are instructed to only yield those trees in a good state of vitality (DGRF, 2006 b). However, this does not explain why the very young trees (DBH class 0) show a mortality of 17 %, which is 5 % higher than the average mortality. This observation might add to the suggestion made above, that young trees are more vulnerable to the disease. The highest mortality rates however, occurred in DBH classes 5 and 6 (35 % and 25 %). This fact has extremely serious economic consequences, because in the field of cork harvest, it is these classes which promise the highest profits under normal conditions.

Symptoms and topographical aspects

Throughout the whole distribution, a linear increase of the crown symptoms- water sprouts and dead branches- could be proved in association with rising damage classes. The significance was tested with $R^2= 0,9242$ for water sprouts and $R^2= 0,9842$ for dead branches (see annex 11). Taking a closer look at these shares for the DBH classes mentioned before, it can be seen that although DBH class 5 features the highest mortality rate, the total sanitary state is worst in DBH class 6, containing only trees with at least moderate damage. This proportion is reflected by the crown symptoms: with a quotient of water sprouts/ dead branches of 0,77 in DBH class 6 compared to 0,39 in DBH class 5, the trees in class 6 react with an evidently higher production of water sprouts in relation to the amount of dead branches. Further, the data imply that mature trees, independently from their damage class (Dc 2 for DBH class 7 and Dc 3 for DBH class 9) tend to develop higher water sprout shares. However, it must be borne in mind, that both DBH class 7 and 9 contained only one individual and are therefore are too small as a reference quantity.

The high standard deviation for the production of water sprouts (up to 36 % in Dc 4) leads to the assumption that on the single tree level very different states of vitality exist. The differences in standard deviation of branch dieback on the one hand, and the formation of water sprouts, as a struggle for survival, on the other, increase linearly with rising state of damage,with a significance of $R^2 = 1,0$ (see annex 12). Further research on this task might help to understand why some trees manage to resist the disease longer than others. Beside genetic attributes, factors like the development of the root system (HARRACHI, 2000), the specific site (DGRF, 2006 b) and the supply of mycorrhiza (MARX and DAVEY,1969) might be decisive. A better understanding of these factors could provide an important key in how to oppose the disease.

The assessment of the trunk symptoms revealed that 36,4 % of all trees showed characteristic tongue shaped exudations in the root base region. This number is evidently higher than the one presented by BRASIER et al (1993), who only observed these exudations on 5- 10 % of the declining trees. However, if their observation is interpreted thus that affected trees may, but not necessarily need to produce exudations, the result of this survey might add, that the manifestation of exudations may vary in frequency. The shares of tongue shaped exudations grew polynomially together with the damage classes with a significance level of $R^2 = 0,96$ (annex 7). A pronounced occurrence of tongue-shaped exudations could be related to trees with shares of dead branches above 80 %. This would imply that these symptoms are to be found preferably in an advanced state of decline and indicate near- death. BRASIER et al. (1993) associated this ‘stem girdling’ to moist sites and a rather rapid procedure of the disease. This hypothesis can be supported, as on the dry and south exposed northern hillside no exudations at the root base were determined on the dead trees at all (annex 9). This leads towards a real problem, because apart from the rather unspecific crown dieback these root based exudations are the only specific symptom for *Phytophthora*. And as further the isolation from the soil is very problematic on these dry sites (Brasier,1992, see above), there is no real evidence that *B. mediterranea* might not be the only agent on this hill. Still the pathogen must have got into the valley somehow and as it spreads with the water it is most likely that it came from the sites above, where, according to the landlord (Da Silva Lução, verbal communication, 26.II. 2007), the first dead trees were observed.

A polynomial increase of damage could be demonstrated within a 10 metres range of the waterlines with a significance of $R^2 = 0,98$. This confirms the observations made by Brasier et al. (1993) and Moreira and Martins (2005) that *P. cinnamomi* occurs more frequently in association with brooklets,valleys and depressions.

Additional Remarks

To what extent the appearance of phyllophagous insects (*Rhynchaeus quercus, Phyllobius argentatus* and caterpillars of *Tortrix viridana*) will influence the decline of the trees cannot be predicted yet. Still, Harrachi (2000) qualifies them as primary agents and Brasier (1996) highlights that insect attacks may accelerate the procedure of the disease and even initiate sudden death. Defoliation by *T. viridana* is a factor in the decline of oaks in Germany (Altenkirch and Hartmann, 1987). Hence an aggravation of the process can be suspected from this aspect as well.

Finally, a hypothesis shall be formulated, which may be of interest in the practical control of the disease: *P. cinnamomi* disposes of very effective abilities in regard to its dispersal and survival (see chapter 2.3.1). However, the survey in the laboratory revealed indirectly that a weak point seems to be its competitive power. All the samples examined by Prof. Dr. Kehr and myself were quickly (within 10 days) outgrown by other fungi or bacteria. This means that if the pathogen does not manage to find a suitable host within a comparatively short period of time, it can not reproduce itself. This hypothesis is sustained by the findings of Shearer and Tippett (1989 in Moreira and Martins, 2005) in their survey of *Eucalyptus* forests in Jarrah, stating that the high presence of *P. cinnamomi* was favoured by low populations of antagonistic soil microflora. Marx and Davey (1969) specify the effects of an antagonistic microflora for the example of mycorrhiza and by this show a possible method of a biological disease control.

Concluding, it has to be noted that because of the high subdivision of the data, the reference groups in some sub- classes turned out to be rather small. For this reason, a bigger test group can be recommended for further surveys. It should also be borne in mind, that additional pathogens (e.g. *Diplodia mutila*) might play a part in other sites. The results of this assessment will have to be combined with the outcomes of other research projects to develop a comprehensive perspective for the future.

6 Recommendations for disease management

6.1 Diagnosis and monitoring

- Because of the comparatively small management units (farm level), the dimensions of the single decline foci are hard to assess. Bigger management units are necessary to acquire a working perspective on the respective plot sizes. In this context, the Forestry Intervention Zones(Zonas de Intervençaő Florestal) might be the right instrument.
- So far, the survey of the area infested in Portugal is limited to scientific spot- checks. These results should be gathered and mapped together with the outcome of the forest inventory (IFN). In blind spots data should be supplemented. Infestation maps could be set up containing the outlines of the foci, watersheds and waterlines. These maps and a regular survey would form the base for a specific monitoring system.
- Within this framework more resistant individuals could be identified and used for breeding.

The formation of a monitoring system is aimed at by the annexes I a-d and II a of the Programme for the Defence of *Q. suber* Populations (PDPS) (MINISTRO DA AGRICULTURA, DESENVOLVIMENTO RURAL E PESCAS, 2003).

6.2 Prophylaxis

- Information: An information of the local population is indispensable. Many of the farmers still do not know what kind of agent causes the disease on their land, nor how to avoid or to oppose it. An information centre should be formed.
-Inhibition of further dispersal and hygiene: Driving on forest soils with heavy machinery and especially the technique of the 'limpeza' needs to be restricted. Not only is this technique one of the most likely anthropogenic distribution modes for *Phytophthora*-contamined soil particles (KLIEJUNAS and KO, 1976), but also soil compression leads directly to decreasing oxygen levels and thus increases the susceptibility to the disease (JAKOBS,1991, in MAC DONALD, 1994). The machinery drivers should be trained to recognize decline foci and avoid them or- if this is not possible- to properly clean their tyre treads afterwards. Irrigation from infested areas needs to be stopped. The routes of water distribution should be controlled, e.g. with the method ELISA (Enzyme- Linked Immunosorbent Assay), used by ALI- SHITAYEH et al. (1991). It is important that clean seedlings are provided for afforestation projects. Nurseries can avoid a spread of the

disease by using sterile soil, chemicals (Fosetyl Al, metalaxyl, etridiazol), clean stock, and sloping beds of coarse, gravel on which to place the pots (REUTER, C.; 2005, internet 3).

6.3 Disease control

As *B. mediterranea* is a secondary/ weakness parasite, it will be useful to concentrate the efforts on *Phytophthora* as the primary pathogen. However cutting and burning of infested material are a way to reduce the infection sources of *B. mediterranea* (OKLAHOMA STATE UNIVERSITY, 2007, internet 5).

- Classical control by the extraction of host plants is considered very difficult to realise in the case of *P. cinnamomi*, because other maquis species (like *Cistus* spp., *Ulex* spp., *Calluna vulgaris*) are infected as well and serve as a reservoir (MOREIRA et al., 2006).
-Alternative species: In highly contamined soils, alternative tree species such as *Pinus pinaster* are infected as well (MOREIRA and MARTINS, 2005). From Germany, however, it is known that *Fraxinus excelsior* is not susceptible to *Phytophthora* (KEHR, 2004). If this observation can be confirmed for *Fraxinus angustifolium*, this might be an interesting alternative species in moist valleys.
- Breeding of resistant plants: Research in order to find more resistant plants is being carried out since 2004 in a joint project between the University of Huelva, the University of the Algarve and the Estaçaő Agronómica Nacional -INIAP (MOREIRA et al., 2006). Good communication between research institutes and local technicians will be helpful for the discovery of more resistant genetic material.
- Antifungal treatment: FERNANDEZ- ESCOBAR et al. (1999) developed a method to treat diseased oaks with pressurised injection capsules which allow the introduction of antifungal substances directly into the tree without environmental contamination. This method is applied in some areas of Portugal.
- Mycorrhiza: In their studies on pine roots, MARX and DAVEY (1969) exemplified the important role ectomycorrhizal fungi can play in the protection of their symbiont trees against the attack of *P. cinnamomi*, acting as a mechanical barrier and competing for nutrients and space. MARX (1972) documented the synthesis of antibiotic or inhibitory substances affecting root pathogens for more than 90 ectomycorrhizal fungi. VROT and GRENTE (1985) identified several mycorrhizal fungi (e.g. *Cantharellus* sp.) to be effective antagonists to *P. cinnamomi* on *Castanea sativa*. These experiments might form the basis for a biological control of the disease. Oak stands could be inoculated with the symbiont's

mycelium, thus improving the trees vitality on the one hand and providing an additional income for the farmers on the other. This task is also included in the necessities of investigations formulated in annex II a of the PDPS (MINISTRO DA AGRICULTURA, DESENVOLVIMENTO RURAL E PESCAS, 2003).

-Drainage: The use of drainage ditches (MOREIRA and MARTINS, 2005) might be a possibility to protect single stands.

6.4 Competent advice and local support

Afflicted farmers should be counselled by expert personnel in how to fight the disease. In legitimate cases, farmers should be supported by material or financial means, because it is not only a problem of single stands but a question of public interest to inhibit the further spread of the disease.

These two points are considered by the PDPS in the annex I c, which provides the formation of a technical corps for this purpose (MINISTRO DA AGRICULTURA, DESENVOLVIMENTO RURAL E PESCAS, 2003). With the PDPS Portugal certainly took a step in the right direction and it will be interesting to watch how it is implemented in the future.

7 Summary

Since the 1970ies, there has been increasing oak mortality occurring in Portugal, leading to a drastic reduction of the cork production (DGRF, 2006 a). In 1992, BRASIER (1992) determined *Phytophthora cinnamomi* to be a major contributory agent to the disease. Since then, this pathogen has been identified on many different sites throughout Iberia (BRASIER et al, 1993; MOREIRA and MARTINS 2005; MOREIRA et al., 2006). *P. cinnamomi* is a highly virulent soil- and water- borne pseudofungus, known for causing root rot on its host plants (BRASIER et al. 1993).

In this work, a plot in the Baixo Alentejo Region in south- western Portugal was surveyed . The aims were to determine if this pathogen is involved in the increased oak mortality here as well, and which other factors contribute to the disease. For this purpose, soil- and tissue-samples were gained from the plot and examined for possible pathogens. Two species of *Phytophthora* (*P. cinnamomi* and *P. syringae*), *Biscogniauxia mediterranea* and *Pythium* spp. could be isolated from these samples (BIOLOGISCHE BUNDESANSTALT, 2007). Further, the formation of the symptoms was assessed in order to recognize a pattern for infestation, as well on the trees themselves, as in a topographical respect. 250 trees were recorded with their GPS coordinates and judged by their damage class, crown symptoms and exudations. A digital map of the area was created. The evaluation of the data revealed that, although dead trees could be found all over the territory, a concentration was observed near the waterlines. A second focus of decline was found on the south facing hillside, with a high participation of *B. mediterranea.* The highest mortality rates occurred on mature trees (DBH classes 5 and 6), but a raised level could also be observed for the very young trees (DBH class 0).

With rising states of damage, a linear growth of the crown symptoms (dead branches and water sprout- shares) was encountered, whereas the root base symptoms (exudations) showed an almost polynomial growth. An enhanced occurrence of root base symptoms could be associated with shares of dead branches above 80 %.

Finally, a set of recommendations for disease management within infested areas was outlined.

Zusammenfassung

In Portugal ist seit den 70er Jahren eine erhöhte Eichensterblichkeit zu beobachten, was zu einer drastischen Verminderung der Korkproduktion seit dieser Zeit führte (DGRF, 2006 a). 1992 entdeckte BRASIER (1992), daß *Phytophthora cinnamomi* als einer der maßgeblichen Faktoren an der Erkrankung beteiligt ist. Seitdem wurde dieses Pathogen auf vielen verschiedenen Standorten in ganz Iberien nachgewiesen (BRASIER et al., 1993; MOREIRA und MARTINS 2005; MOREIRA et al., 2006).). *P. cinnamomi* ist ein hochvirulenter, boden- und wasserbürtiger Pseudofungus, der dafür bekannt ist, eine Wurzelfäule an seinen Wirtspflanzen zu verursachen (BRASIER et al. 1993).

In der vorliegenden Arbeit wurde eine Fläche in der Region Baixo Alentejo im Südwesten Portugals untersucht. Dabei war die Zielsetzung, herauszufinden, ob dieser Erreger auch hier an der erhöhten Eichensterblichkeit beteiligt ist, und welche anderen Faktoren zu der Erkrankung beitragen. Zu diesem Zweck wurden im Untersuchungsgebiet Boden- und Gewebeproben gewonnen und auf mögliche Erreger geprüft. Zwei *Phytophthora*– Arten (*P. cinnamomi* und *P. syringae*), sowie *Biscogniauxia mediterranea* und *Pythium* spc. konnten aus diesen Proben isoliert werden (BIOLOGISCHE BUNDESANSTALT, 2007). Desweiteren wurde die Ausprägung der Symptome beurteilt, mit dem Ziel, ein Befallsmuster, sowohl an den Bäumen selbst, alsauch in topografischer Hinsicht zu erkennen. 250 Bäume wurden mit ihren GPS- Koordinaten eingemessen und nach Schadstufe, Kronensymptomen und Schleimflussmerkmalen bewertet. Es wurde eine digitale Landkarte des Gebiets erstellt. Obwohl abgestorbene Bäume über die gesamte Probefläche verteilt vorkamen, ergab die Auswertung der Daten, daß eine Häufung in Nähe der Wasserlinien zu beobachten ist. Ein zweiter Krankheitsherd, der eine hohe Beteiligung von *B. mediterranea* aufwies, konnte an der südexponierten Bergflanke ausgemacht werden. Die höchsten Sterblichkeitsraten traten an ausgewachsenen Bäumen (Stärkeklassen 5 und 6) auf, jedoch konnte ein erhöhtes Niveau auch für die ganz jungen Bäume (Stärkeklasse 0) ausgemacht werden. Mit zunehmendem Schadstatus ging ein linearer Anstieg der Kronensymptome (Totäste und Wasserreiser) einher, während die Symptome im Bereich der Wurzelanläufe (Schleimfluss) ein annährend polynomisches Wachstum zeigten. Ein verstärktes Auftreten von Symptomen an den Wurzelanläufen konnte mit Totastanteilen von über 80% in Verbindung gebracht werden.

Schließlich wurde ein Behandlungskonzept für befallene Gebiete entworfen.

Sumario

Desde os anos 1970, houve um grande aumento de mortalidade de sobreiros em Portugal, tendo como consequência uma drástica redução de produção de cortiça (DGRF, 2006 a). Em 1992, BRASIER (1992) determinou o agente *Phytophthora cinnamomi* de ser o maior contributo para tal doença. Desde então, o patogénio tem sido identificado em muitos locais diferentes pela Iberia fora (BRASIER et al,1993; MOREIRA e MARTINS 2005; MOREIRA et al., 2006). *P. cinnamomi* é um fungo pseudo altamente virulento com origem na agua e solo, conhecido pelo apodrecimento de raízes na sua planta mãe (BRASIER et al. 1993). Neste trabalho, uma área na região do baixo Alentejo no sudoeste de Portugal foi pesquisada. Os objectivos eram de determinar se o patogénio está envolvido no aumento de mortalidade de sobreiros nesta região, e de determinar quais eram os outros factores que contribuíram para tal doença. Para o efeito, amostras de solo e de tecido vegetal foram retiradas da área de estudo e examinado por eventuais patogénios. Duas espécies de *Phytophthora (P. cinnamomi* e *P. syringae), Biscogniauxia mediterranea* e *Pythium spp.* foram isoladas das amostras (BIOLOGISCHE BUNDESANSTALT, 2007).

Adicionalmente, a formação de sintomas foi avaliada em ordem a poder reconhecer o padrão de infestação, tal como foram as próprias arvores, em respeito topográfico. 250 arvores foram anotadas com as suas coordenadas de GPS e avaliadas pela sua degrau de estrago, sintomas de coroa e exsudações. Um mapa digital da área de estudo foi criado. A avaliação da data revelou que, mesmo podendo se encontrar arvores mortas no terreno, uma concentração de mortalidade foi anotada perto de linhas de agua. Um decrescimento foi encontrado em faces sul de montes, com uma elevada presença de *B. mediterrane*a. A maior taxa de mortalidade tem ocorrido em arvores adultas (diâmetro classe 5 e 6), mas um aumento foi observado em arvores muito jovens (diâmetro classe 0). Com um aumento de estados de estrago, um crescimento linear de sintomas de coroa (ramos mortos e rebentos de agua) foram encontradas, apesar os sintomas de base nas raízes (exsudação) tem mostrado um aumento exponencial. Um crescimento de sintomas de base nas raízes, poderá ser associado com taxas de ramos mortos acima de 80%.

Finalmente, uma serie de recomendações para o tratamento técnico da área infestada foram propostos.

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Maps

Annexes