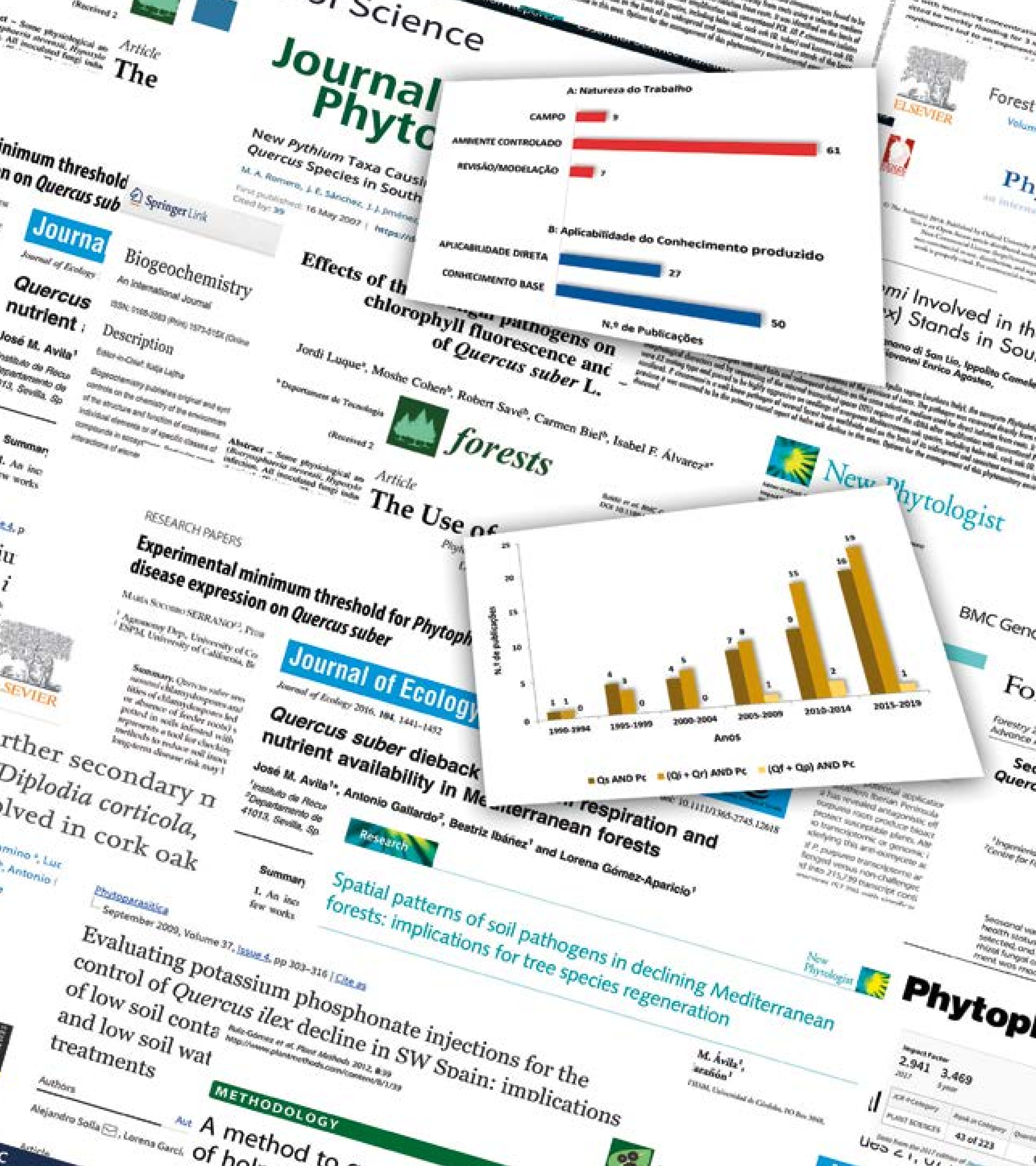

Phytophthora cinnamomi em espécies de *Quercus* e do sobcoberto de montados e *dehesas*. Análise de publicações científicas.

Phytophthora cinnamomi* em espécies de *Quercus* e do sobcoberto de montados e *dehesas.

Análise de publicações científicas.

Manuel Trindade, Filipe Costa e Silva, Ana Cristina Moreira,

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PRODEHESA MONTADO
Proyecto de Cooperación Transfronteriza para la Valorización Integral de la Dehesa-Montado

iniav Instituto Nacional de Investigação Agrária e Veterinária, I.P.

CICYTEX CENTRO DE INVESTIGACIONES CIENTÍFICAS Y TECNOLÓGICAS DE EXTREMADURA

unac União da Floresta Mediterrânica

FICHA TÉCNICA

Autores

Manuel Trindade (INIAV I.P.)

Filipe Costa e Silva (ISA)

Ana Cristina Moreira (INIAV I.P.)

Conceição Santos Silva (UNAC)

Enrique Cardillo (CICYTEX)

Teresa Soares David (INIAV I.P.)

Editor

INIAV I.P.

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RESUMO

Este documento pretende contribuir para a análise da produção científica (1990 – 2019), sob a forma de artigos com revisão por pares, sobre *Phytophthora cinnamomi* em espécies de *Quercus* e do sobcoberto de montados/*dehesas*. Os problemas decorrentes do declínio dos montados e *dehesas*, com consequências a nível económico, ambiental e social que se repercutem na sua sustentabilidade, têm suscitado grande preocupação por parte de produtores, técnicos e gestores florestais.

O conhecimento científico acumulado é fundamental para sustentar medidas eficazes de prevenção e controlo deste agente patogénico que interage em ambiente natural com outros fatores bióticos e abióticos.

Com base numa pesquisa bibliográfica realizada na plataforma *WoS* sobre *Phytophthora cinnamomi* em *Quercus* spp. e em espécies do sobcoberto de montados e *dehesas*, e seguindo critérios previamente definidos, obteve-se um conjunto de 79 publicações científicas que foram analisadas e enquadradas por área temática. Elaborou-se uma base de dados com os artigos inventariados por área temática dominante e sintetizaram-se os principais resultados das publicações.

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ANEXO III – Nomes científicos e comuns das espécies vegetais referidas no texto.

1. INTRODUÇÃO

O montado e a *dehesa* são sistemas agrosilvopastoris com grande importância económica, social e ecológica em Portugal e Espanha onde ocupam 1,1 e 3,5 milhões de hectares, respetivamente (Pulido e Picardo, 2010). As espécies arbóreas dominantes nos montados são o sobreiro (*Quercus suber*) e a azinheira (*Quercus rotundifolia = Q. ilex* subsp. *ballota*). O sobreiro ocupa uma área de 720 mil ha e a azinheira 349 mil ha representando, respetivamente, 22,3% e 10,8% da área florestal portuguesa (ICNF, 2019). Nas *dehesas* a espécie arbórea dominante é a azinheira, embora ocorram também sobreiros e outras espécies do género *Quercus* como o carvalho cerquinho (*Quercus faginea*) e o carvalho negral (*Quercus pyrenaica*).

Desde meados do séc. XX vêm sendo reportadas situações de declínio dos montados e *dehes*as, com particular incidência desde 1980. O declínio tem sido atribuído a fatores abióticos e bióticos. No entanto, diversos trabalhos realizados na Península Ibérica dão particular relevância ao agente fitopatogénico *Phytophthora cinnamomi*. A nível mundial, é também bem documentada a associação do género *Phytophthora* spp. a graves problemas fitossanitários em diversas espécies florestais como *Quercus rubra* e *Quercus robur* (Brasier, 1999), *Castanea sativa* (Pimentel, 1947), *Eucalytpus marginata* (Davison, 2018), *Eucalyptus fraxinoides* e *E. dunnii* (Maseko *et al*., 2007), e *Pinus taeda* (Coyle *et al*., 2015).

Devido à importância dos montados e *dehesas*, e à crescente preocupação com a sua sustentabilidade, é importante analisar o conhecimento que tem sido produzido em publicações científicas sobre estes ecossistemas, nomedamente as que relacionam *P. cinnamomi* com as espécies de *Quercus* e do sobcoberto que os integram. Esta análise poderá contribuir para sustentar, com base científica, a adoção de medidas de prevenção e controlo da doença originada pelo oomiceta *P. cinnamomi*.

2. OBJETIVOS

Neste trabalho pretende-se :

- Inventariar as publicações científicas, com revisão por pares, produzidas sobre *Phytophthora cinnamomi* em *Quercus* spp. e em espécies do sobcoberto de montados/*dehesas*;
- Analisar a evolução temporal do número de publicações e a sua distribuição geográfica;
- Analisar a distribuição das publicações por áreas temáticas dominantes;
- Construir uma base de dados dos artigos inventariados, agrupados por área temática, incluindo critérios de qualidade dessas publicações;
- Apresentar uma síntese dos principais resultados das publicações por área temática.

3. METODOLOGIA

Como ferramenta de base para a elaboração deste trabalho foi utilizada a plataforma *Web of Science* (*WoS*), da *Clarivate Analytics* (antes *Thomson Reuters*) (http://apps.webofknowledge.com/). Esta é uma plataforma de bases de dados de referências bibliográficas e de índices de citações. Permite, através de recurso ao *Journal Citation Reports* (JCR), avaliar e comparar publicações científicas periódicas com base na determinação de índices bibliométricos, como o fator de impacto (IF, *impact factor*) e o nº de citações. O IF é um indicador da relevância da publicação (por ex. IF (2017) é o nº médio de citações/artigo, dos artigos publicados nos últimos dois anos). O nº de citações é um indicador de relevância do artigo, apesar de nos artigos mais recentes não ser possível uma diferenciação através do nº de citações. A *WoS* permite, entre outros aspetos, a pesquisa por ocorrência de palavras selecionadas, agregando publicações científicas com diversos formatos: artigos em revistas científicas internacionais com arbitragem científica, capítulos de livros, artigos em conferências, *abstracts* de reuniões, notícias, etc. A caixa de pesquisa básica permite a introdução de termos únicos, expressões complexas ou vários termos separados por operadores booleanos (“AND”, “OR”, “NOT”).

A pesquisa efetuada englobou todas as publicações presentes na plataforma *WoS* entre 1990 e a data de consulta - 11 de maio de 2019. Os termos/expressões selecionados como critérios de pesquisa foram procurados no descritor “*topic*” da plataforma que contempla o Título, o Resumo, as Palavras-chave fornecidas pelo autor e as *Keywords Plus*® (Palavras-chave atribuídas pela plataforma *WoS*).
Consideraram-se as publicações devolvidas pela pesquisa que preenchiam os seguintes critérios:

- Incluir, no descritor “*topic*”, os termos/expressões de pesquisa (ex: *Quercus suber*, *Phytophthora cinnamomi*);
- Ter fator de impacto atribuído e corresponder apenas a artigos científicos com revisão por pares.

A pesquisa englobou duas fases: numa primeira fase, publicações científicas sobre *Phytophthora cinnamomi* (Pc) em *Quercus* spp. e, numa segunda fase como complemento, publicações sobre *Phytophthora cinnamomi* relacionadas com: 1) algumas espécies arbustivas/herbáceas do sobcoberto de montados e *dehesas* (*Lupinus albus, Lupinus luteus*, *Calluna vulgaris, Cistus ladanifer, Cistus salviifolius, Cistus populifolius, Genista triacanthos, Myrtus communis*, *Helichrysum stoechas, Lavandula stoechas, Daphne gnidium, Erica lusitanica, Erica arborea, Phlomis purpurea*); 2) termos equivalentes ou relativos a tipos de vegetação do sobcoberto (*understorey*, *understory*, *herbaceous*, *shrubs*).

4. RESULTADOS DA PESQUISA

Na 1ª parte deste trabalho (4.1) apresenta-se o universo de registos devolvidos pela plataforma *WoS* para publicações sobre *Phytophthora cinnamomi* em *Quercus* spp. É feita a análise da evolução temporal da produção científica, da distribuição geográfica das publicações baseada na afiliação das instituições a que pertencem os autores, da natureza do trabalho desenvolvido e aplicabilidade mais ou menos direta dos resultados gerados, e da distribuição das publicações pelas áreas temáticas definidas.

Na 2ª parte (4.2) apresentam-se os resultados da pesquisa relativa a publicações sobre *P. cinnamom*i em espécies do sobcoberto.

4.1 - PUBLICAÇÕES SOBRE *Phytophthora cinnamomi* em *Quercus* spp.

No Quadro 1 apresenta-se o número de publicações devolvido pela plataforma *WoS* de acordo com os termos/expressões pesquisados. Verifica-se que no universo de publicações sobre o género *Quercus*, o número de artigos científicos em azinheira é muito maior que em sobreiro (14,9% vs. 6,6%, respetivamente). Este facto poderá refletir a mais ampla distribuição geográfica da azinheira na Península Ibérica.

Das 178 publicações devolvidas pela plataforma *WoS*, relativas à pesquisa para "*Quercus*" e "*Phytophthora cinnamomi*", verificou-se que cinco correspondiam a atas de conferências não publicadas em forma de artigo e 64 não incluíam, no título, resumo ou palavras-chave do autor, os termos/expressões pesquisados. A inclusão de *keywords plus* no descritor "*topic*" permite que sejam devolvidas publicações em que os termos/expressões pesquisados aparecem apenas na introdução, discussão ou bibliografia. Dos 109 artigos remanescentes de *P. cinnamom*i em *Quercus* spp. foram ainda excluídos da análise 32 artigos relativos apenas a espécies de *Quercus* que não integram os montados/*dehesa*s: *Q. agrifolia*, *Q. alba*, *Q. canariensis*, *Q. cerris*, *Q. elliptica*, *Q. frainetto*, *Q. hartwissiana*, *Q. ithaburensis*, *Q.* glaucescens, *Q. glaucoides*, *Q. macranthera*, *Q. magnoliifolia*, *Q. montana*, *Q. palustris*, *Q. pendunculari*s, *Q. petraea*, *Q. robur*, *Q. rubra, Q. salicifolia, Q. virgiliana* e *Q. vulcanica*.

O universo de publicações analisadas sobre *P. cinnamom*i em *Quercus* spp. de montados/*dehesas* ficou assim restringido a 77, *i.e.*, 4,4% do total de publicações sobre *Phytophtora cinnamomi* (1754). É de salientar que 21 destes 77 artigos abordam simultaneamente mais do que uma espécie de *Quercus* spp. (*i.e.*, o somatório do número de artigos por espécie excede 77).

Quadro 1 – Número de publicações, e valores percentuais, em *Quercus* spp., em *Quercus* spp. de montados e *dehesas*, em *Phytophthora cinnamomi*, e em cada uma das conjugações de termos/expressões analisados (pesquisa em 11/05/2019 na plataforma *WoS*)

Termos/expressões de pesquisa	**Nº de publicações**	**%** (1)
Quercus	20513	
Quercus suber	1359	6,6
Quercus ilex **OR** *Quercus rotundifolia*	3057	14,9
Quercus faginea **OR** *Quercus pyrenaica*	591	2,9
Phytophthora cinnamomi	1754	
Pc em *Quercus* spp. dos montados/*dehesas*	77	**4,4** (2)
Quercus suber **AND** *Phytophthora cinnamomi*	41	**53,2** (3)
(*Q. ilex* **OR** *Q. rotundifolia*) **AND** *Phytophthora cinnamomi*	51	**66,2** (3)
(*Q. faginea* **OR** *Q. pyrenaica*) **AND** *Phytophthora cinnamomi*	4	**5,2** (3)

(1) % do nº de publicações em relação ao total de 20513 publicações sobre o género "*Quercus*"
(2) % do nº de publicações em relação ao total de 1754 publicações sobre "*Phytophthora cinnamomi*"
(3) % do nº de publicações em relação ao total de 77 publicações sobre Pc em "*Quercus*"

4.1.1 - EVOLUÇÃO TEMPORAL DA PRODUÇÃO CIENTÍFICA

A análise da evolução temporal do número de publicações sobre *P. cinnamom*i em *Quercus* spp. permite observar uma tendência crescente entre 1990 e 2019 (11/05/2019), em "*Q. suber*" e em "*Quercus ilex/Quercus rotundifolia*" (Figuras 1 e 2). Neste período foram apenas publicados quatro artigos científicos em *Q. faginea* e *Q. pyrenaica*, espécies com menor representação nas *dehesas*.

A Figura 2 evidencia um aumento considerável, a partir de 2010/2011, de publicações sobre *P. cinnamom*i em "*Quercus ilex/Quercus rotundifolia*" relativamente a "*Quercus suber*", refletindo o trabalho produzido por instituições espanholas.

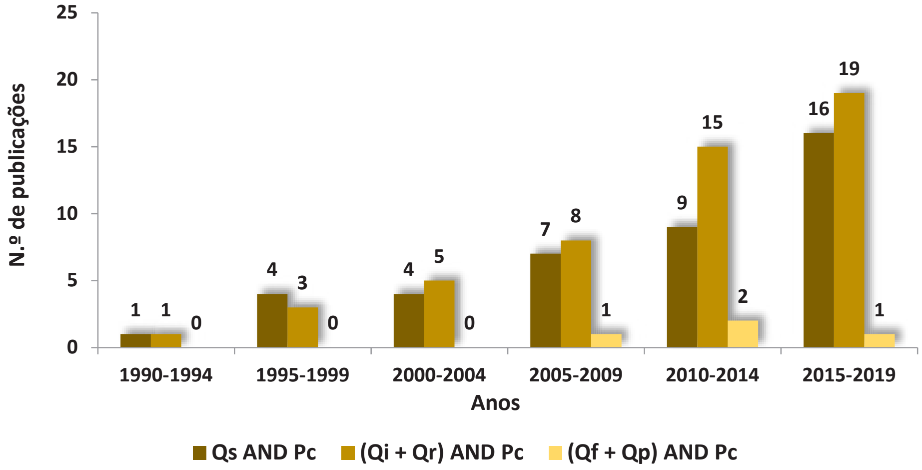

Figura 1 – Evolução do número de publicações (por períodos de 5 anos) sobre *Phytophthora cinnamomi* (Pc) em *Quercus* spp. (Q AND Pc): *Q. suber* (Qs) AND Pc; (*Q. ilex* (Qi) OR *Q. rotundifolia* (Qr)) AND Pc; (*Q. faginea* (Qf) OR *Q. pyrenaica* (Qp)) AND Pc.

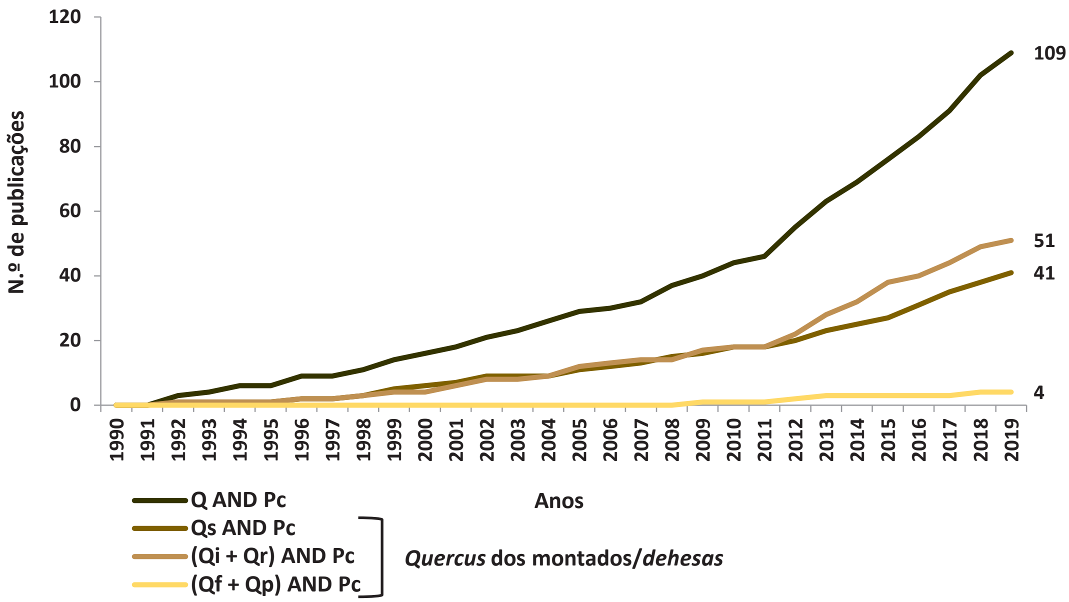

Figura 2 – Evolução do número de publicações (valores anuais acumulados) sobre *Phytophthora cinnamomi* (Pc) em *Quercus* spp. (Q AND Pc): *Q. suber* (Qs) AND Pc; (*Q. ilex* (Qi) OR *Q. rotundifolia* (Qr)) AND Pc; (*Q. faginea* (Qf) OR *Q. pyrenaica* (Qp)) AND Pc.

4.1.2 - DISTRIBUIÇÃO GEOGRÁFICA DA ORIGEM DAS PUBLICAÇÕES (AFILIAÇÃO INSTITUCIONAL)

Nesta análise agruparam-se as publicações de acordo com o país de afiliação institucional dos seus autores, considerando-se três zonas geográficas: Península Ibérica (Portugal e Espanha), Bacia Mediterrânica (Portugal, Espanha, França e Itália) e outros países do Mundo (Suécia, Holanda, Reino Unido, Suíça, Alemanha, EUA e África do Sul).

De acordo com a afiliação institucional dos vários autores agruparam-se as publicações em 4 quatro sub-grupos (Quadro 2):

- o 1.º autor tem afiliação institucional a países não incluídos na Bacia Mediterrânica, designado por Resto do Mundo (**RM**);
- o 1.º autor tem afiliação institucional a um país da Bacia Mediterrânica, que não inclui Portugal ou Espanha, designado por Resto da Bacia Mediterrânica (**RBM**);
- todos os autores têm afiliação apenas a instituições portuguesas (**PT**) ou o 1º autor tem afiliação a instituições portuguesas mas os restantes autores podem não ter, englobando os casos (**PT+ES**) e (**PT+RBM/RM**);
- todos os autores têm afiliação apenas a instituições espanholas (**ES**) ou o 1º autor tem afiliação a instituições espanholas mas os restantes autores podem não ter, englobando os casos (**ES+PT**) e (**ES+RBM/RM**).

Quadro 2 – Número de publicações sobre *Phytophthora cinnamomi* em *Quercus* spp. de montados e *dehesas*, por sub-grupos de afiliação das instituições a que pertencem os autores.

Afiliação do 1.º Autor	N.º de publicações			
PT	PT	PT+ES	PT+RBM/RM	**Total**
	10	3	1	14
ES	ES	ES+PT	ES+RBM/RM	
	37	5	8	50
RBM	9			9
RM	4			4

PT: Portugal; **ES**: Espanha; **RBM**: Resto da Bacia Mediterrânica; **RM**: Resto do Mundo

Portugal, para além das co-autorias com Espanha, publicou artigos científicos com autores de instituições suecas e inglesas. Espanha, para além de co-autorias com Portugal, publicou artigos científicos com autores de instituições italianas, alemãs, suíças, holandesas, inglesas, suecas, norte americanas e sul africanas.

Dos países do RBM apenas França e Itália têm publicações como 1º autor. No entanto, enquanto a maioria das publicações italianas envolve colaborações internacionais (Espanha, Alemanha e EUA), apenas uma publicação francesa envolve colaboração internacional (com Marrocos).

Do Quadro 2 ressalta que Espanha é o país com maior número de publicações (50), representando 64,9% do total das 77 publicações em análise, e que Portugal tem cerca de 18,2% (14 publicações). Em conjunto Portugal e Espanha foram responsáveis por 83,1% da produção científica (64 publicações). O maior número de publicações por instituições espanholas explica também o maior nº de publicações em *Q. Ilex/Q. rotundifolia* relativamente a *Q. suber* (referido em 4.1.1)

4.1.3 - NATUREZA E APLICABILIDADE DOS RESULTADOS

A análise da natureza do trabalho desenvolvido nas publicações - abordagem mais teórica, trabalho realizado em condições controladas (laboratório ou estufa), ou trabalho realizado em campo, foi feita agrupando as publicações em três sub-grupos:

- Publicações apenas com trabalho de campo. São exemplo os trabalhos que recorrem ao levantamento de dados de natureza fisiológica, pedológica, florística ou climatológica.
- Publicações com trabalho desenvolvido apenas ou maioritariamente em condições controladas. São exemplo os trabalhos no âmbito da biologia molecular ou bioquímica, micologia, desenvolvidos principalmente em laboratório mas podendo ter também componente em campo ou estufa com controlo das condições dos ensaios. Estão também contempladas neste grupo as publicações que, apesar de incluírem uma componente de campo (recolha de material biológico, solos, etc.), decorrem maioritariamente em condições controladas.
- Publicações de revisão ou trabalho de modelação teórica. Incluem trabalhos de revisão bibliográfica ou que se enquadram na área da modelação, sendo essencialmente teóricos.

Para a análise da aplicabilidade prática do conhecimento científico produzido foram considerados dois sub-grupos de publicações: trabalhos que resultam em conhecimento científico prático/mais diretamente aplicável; trabalhos que produzem conhecimento teórico de base. São exemplo do primeiro caso as publicações que abordam o isolamento e idenficação laboratorial de material vegetal recolhido em campo, testes de patogenicidade, ensaios de eficácia de pesticidas ou biopesticidas, ensaios de resistência, etc.; e, do segundo caso, publicações em que se desenvolvem modelos de previsão da expansão de *Phytophthora cinnamomi*, clarificam mecanismos metabólicos e de infeção, testam tecnologias de aplicação laboratorial, etc.

Em 79,2% das publicações (61) o trabalho realizado desenvolveu-se maioritariamente em ambiente controlado sendo muito reduzido o número de publicações em que foi feito apenas trabalho de campo ou de natureza teórica – revisão/modelação (Figura 3A).
Em relação ao tipo de conhecimento gerado, 35,1% das publicações contribuíram com resultados de aplicabilidade direta e 64,9% com conhecimento de base (Figura 3B).

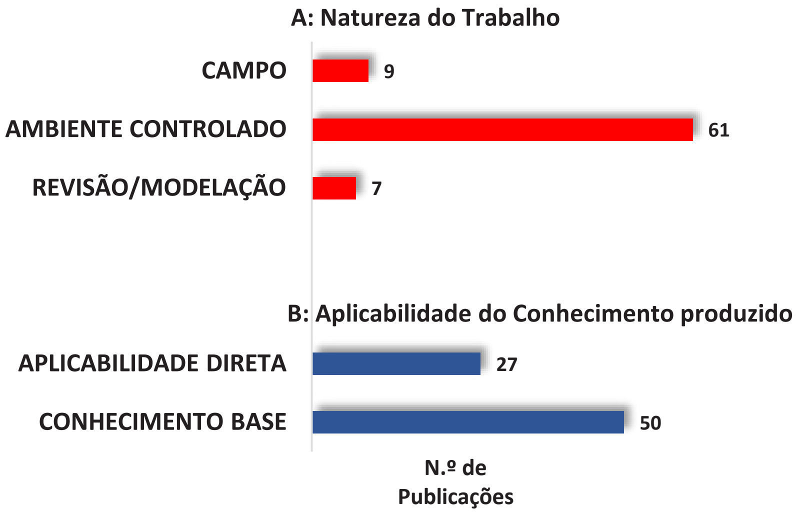

Figura 3 - Número de publicações sobre *Phytophthora cinnamomi* em *Quercus* spp. de montados e *dehesas*, segundo a natureza do trabalho desenvolvido (**A**) e a aplicabilidade do conhecimento científico produzido (**B**).

4.1.4 - DISTRIBUIÇÃO POR ÁREA TEMÁTICA DOMINANTE

Apesar de ser frequente encontrar num mesmo artigo científico uma abordagem a várias áreas temáticas, agruparam-se os artigos sobre *P. cinnamomi* em *Quercus* spp. por área temática dominante, face aos seus objetivos e principais conclusões. Definiram-se seis áreas temáticas:

Fatores Bióticos (Agente Patogénico/Hospedeiro)

Publicações cuja abordagem principal envolve uma componente biótica, quer sobre o agente fitopatogénico em estudo, quer sobre outros agentes de natureza biótica. Incluíram-se, por exemplo, neste grupo artigos sobre fungos da família Botryosphaeriaceae, sobre outros oomicetas como é o caso de *Pythium* sp., ou sobre insetos que constituem importantes pragas em *Quercus* spp. Estas publicações inserem-se no âmbito da Micologia e da Patologia vegetal/florestal e caracterizam não só os agentes patogénicos a nível morfológico, cultural, mas também as condições ótimas de crescimento ou a análise filogenética, resultado de trabalhos no âmbito da biologia molecular. Nesta área temática incluíram-se também publicações em que é dada maior ênfase ao hospedeiro, relativas à descrição dos testes de patogenicidade ou efeitos nos hospedeiros, à ação de compostos envolvidos nos mecanismos de infeção e de defesa, interação com fungos micorrízicos, etc.

Fatores Abióticos

Publicações que abordam fatores que influenciam o desenvolvimento do patogénio, direta ou indiretamente, nomeadamente stresses relacionados com a influência do clima no equilíbrio do ecossistema, como a falta de água, a influência da temperatura na vitalidade das plantas dos montados/*dehesas*, as características edafoclimáticas, etc.

Biologia Molecular/Bioquímica/Fisiologia

Publicações que abordam técnicas de biologia molecular para estudo de genes envolvidos na infeção e na resposta do hospedeiro, estudo de metabolitos secundários de fungos e avaliação da sua toxicidade em espécies de *Quercu*s, respostas fisiológicas do hospedeiro à infeção e sua conservação através de técnicas de micropropagação.

Prevenção e Controlo

Publicações que abordam ou testam a eficácia de medidas de prevenção e/ou combate a *P. cinnamomi*, desde substâncias ativas sintetizadas artificialmente ou extraídas de plantas, influência de nutrientes minerais, ou outros agentes biológicos com efeito antagonista em *P. cinnamomi* (ex. plantas do sobcoberto).

Ecologia

Publicações que tratam o ecossistema montado/*dehesa* duma forma integrada e generalista, abordando temas como o equilíbrio do ecossistema (modelos para reflorestação), gestão e declínio do montado, influência do clima e alterações climáticas, interações populacionais, efeitos ecológicos de doenças, etc.

Solo

Embora esta área temática esteja integrada em "Fatores Abióticos", foi destacada pela importância do solo na disseminação de *P. cinnamomi* e na expressão da doença causada pelo patogénio. Nela se incluíram publicações sobre o efeito da composição física, química e biológica do solo na expressão da doença.

Sobcoberto

Publicações relacionadas com *P. cinnamomi* mas com particular ênfase nas espécies do sobcoberto (culturas agrícolas, espécies arbustivas da vegetação natural, hospedeiras e não hospedeiras de *P. cinnamomi*), relacionadas com o declínio dos montados/*dehesas*, a suscetibilidade à doença ou com o seu combate.

A maior parte das publicações analisadas integra o tema "Fatores Bióticos" (46,7% correspondendo a 36 registos) (Figura 4), quer o agente patogénico (19,5%), quer o hospedeiro (27,2%). Cerca de 11,7% das publicações aborda o tema "Prevenção e Controlo". Apesar da importância do solo na vitalidade do montado e no ciclo de vida de *P. cinnamomi*, apenas cinco publicações (6,5%) dão enfoque ao tema "Solo".

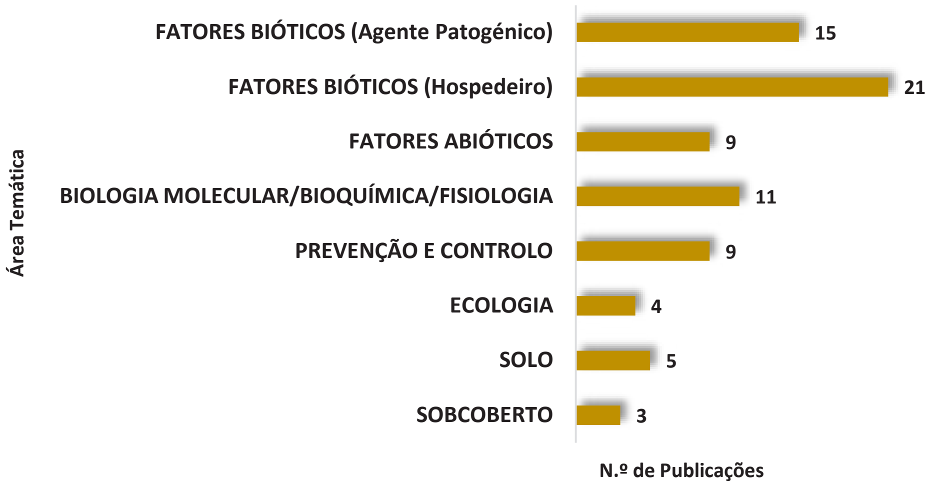

Figura 4 – Número de publicações sobre *Phytophthora cinnamomi* em *Quercus* spp. por área temática dominante.

4.2 - PUBLICAÇÕES SOBRE *PHYTOPHTHORA CINNAMOMI* EM ESPÉCIES DO SOBCOBERTO

A pesquisa realizada sobre *P. cinnamomi* em simultâneo com cada um dos quatro termos/expressões relacionados com sobcoberto - *herbaceous*, *shrubs*, *understorey* ou *understory*, e com as 14 espécies herbáceas/arbustivas frequentes no sobcoberto dos montados/*dehesas* devolveu apenas mais dois artigos (Anexo I - Quadro VIII).

5. BASE DE DADOS DOS ARTIGOS INVENTARIADOS

No Anexo I, Quadros I a VIII, apresenta-se a lista dos 79 artigos devolvidos pela plataforma *WoS* e que cumprem os critérios de pesquisa da 1ª fase, *i.e.*, 77 artigos científicos sobre *Phytophthora cinnamomi* em *Quercus* spp. de montados/*dehesas* (ver 4.1), e da 2ª fase, *i.e.*, 2 artigos científicos sobre *P. cinnamomi* em espécies do sobcoberto de montados/*dehesas* (ver 4.2). Para cada um dos artigos, apresentados por área temática dominante e seriados por ordem cronológica inversa, é dada a referência bibliográfica completa, os indicadores de qualidade da publicação - número de citações em 2019, *impact factor* de 2017 (Clarivate Analytics, 2019), e a *URL*.

Do Anexo II constam os respetivos títulos e resumos.

No Anexo III apresentam-se os nomes científicos e comuns das espécies vegetais referidas no texto.

6. SÍNTESE DOS PRINCIPAIS RESULTADOS DAS PUBLICAÇÕES*

I – Área Temática "Fatores Bióticos – Agente Patogénico"

- As técnicas de biologia molecular vieram complementar as técnicas de isolamento direto na deteção e distribuição de espécies de *Phytophthora*. Os métodos de isolamento direto e com recurso a armadilhas, em azinheiras com e sem sintomas de declínio, revelaram a presença de *Phytophthora cambivora*, *P. cinnamomi*, *P gonapodyides*, *P. megasperma* e *P. pseudocryptogea* em *dehesa*s. O recurso a técnicas de biologia molecular (*TaqMan real-time PCR*) revelou o envolvimento de *P. cinnamomi* e *P. quercina* no declínio de azinheiras em Espanha, com maior frequência de *P. quercina* que de *P. cinnamomi*, quer em ecossistemas seminaturais, quer naturais.

(2)

- Envasaram-se plantas jovens de *Quercus suber* com solo infetado com concentrações crescentes de clamidósporos de *Phytophthora cinnamomi* que se submeteram a encharcamento semanal (três meses) para promover a infeção radicular. O aumento na quantidade de clamidósporos levou a um aumento exponencial na sua capacidade germinativa. Os sintomas radiculares (necroses e/ou ausência de raízes absorventes) foram mais severos nas plantas em solos infetados com pelo menos 61 cfu g^{-1}** que nas plantas em solos não infetados. Este limiar mínimo experimental (em misturas em vasos), para expressão doença em *Q. suber*, poderá permitir conhecer o potencial de infeção de solos infetados e a eficácia de

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* No fim de cada síntese é referido o número de ordem do artigo conforme consta nos Quadros I a VIII do Anexo I.

** cfu: *colony forming unit* (inglês) = unidade formadora de colónias (ufc, português) - unidade de medida usada na contagem do número de microrganismos viáveis de uma amostra (usualmente expresso em cfu g^{-1} ou cfu mL^{-1})

certos métodos de controlo na redução do inóculo. Mesmo em baixas concentrações de clamidósporos (3 cfu g^{-1}) pode ocorrer infeção das raízes.

(4)

- Desde o início do século XX que há registos de mortalidade em *Quercus suber* e *Q. ilex* na Bacia Mediterrânica, com aumento da severidade do declínio a partir de 1980. Desde então, o agente patogénico *P. cinnamomi* tem sido considerado o principal fator de declínio dos montados e *dehesas*. Nesta revisão analisaram-se trabalhos sobre a pesquisa de *P. cinnamomi* em *Q. suber* e *Q. ilex*, testes de patogenicidade em ambiente controlado, a influência de características locais no declínio (retenção de água do solo e expansão das raízes) e metodologias de controlo do patogénio. As observações de campo mostraram que o patogénio está largamente disperso e os testes de patogenicidade revelaram que a suscetibilidade do hospedeiro ao patogénio é moderada se as plantas jovens estiverem em bom estado hídrico. As características do solo influenciam a expansão das raízes e a retenção de água. Avaliou-se a importância relativa de cada um dos fatores de declínio.

(6)

- Apresenta-se uma metodologia para quantificação da infeção e colonização de *Quercus ilex* por *Phytophthora cinnamomi* baseada na captura e tratamento digital de imagens microscópicas, usando *software* simples e acessível, e um conjunto de variáveis que permite quantificar a infeção e o processo de colonização.

(8)

- Inocularam-se plantas de sobreiro com os agentes patogénicos *Botryosphaeria stevensii*, *Hypoxylon mediterraneum* e *Phytophthora cinnamomi*, e avaliou-se o seu efeito na fisiologia e crescimento das plantas infetadas (Catalunha, Espanha). Observou-se uma redução do crescimento diário do caule, da condutância estomática, da temperatura ar-folha e da eficiência fotoquímica. *Botryosphaeria stevensii* e *Phytophthora cinnamomi* foram os patogénios que maior decréscimo originaram nos parâmetros fisiológicos e de crescimento das plantas (decréscimo mais gradual em *P. cinnamomi* que em *B. stevensii*). *H. mediterraneum* originou apenas redução no crescimento e retração do caule.

(14)

- O 1º registo da presença de *Phytophthora cinnamomi* em *Quercus suber* e *Q. ilex* em França ocorreu em 1995-1996 (24 locais analisados) tendo sido confirmada a sua patogenicidade e possível envolvimento no declínio destes carvalhos Mediterrânicos. Verificou-se que *Q. suber* e *Q. ilex* eram menos suscetíveis a *P. cinnamomi* que *Castanea sativa* mas mais suscetíveis que *Q. rubra.*

(15)

Isolamento de oomicetas e fungos em espécies de *Quercus* em Espanha

- Analisou-se o possível envolvimento de espécies de *Phytophthora* no declínio de uma zona dominada por *Quercus ilex* e *Q. faginea* (este de Espanha). Identificaram-se seis espécies de *Phytophthora*: *P. cryptogea*, *P. gonapodyides*, *P. megasperma*, *P. quercina*, *P. psychrophila* e *P. syringae.* Em ensaios realizados em plantas com 1 ano em ambiente controlado, em que *P. cinnamomi foi usado como controlo positivo*, verificou-se que *Q. ilex* era um hospedeiro mais suscetível *a P. cinnamom*i que *Q. faginea* e *P. cinnamomi* o agente patogénico mais agressivo para os dois carvalhos. Verificou-se também patogenicidade de *P. quercina* e *P. psychrophila* em *Q. ilex* e *Q. faginea*.

(7)

- Foram isolados em raízes e solo da rizosfera de sobreiro e azinheira (sul da Península Ibérica) *P. cinnamomi, Pythium spiculum* e *Py. sterilium*. Os ensaios com inoculação de *Py. spiculum* em plantas jovens de azinheira evidenciaram sintomatologia menos agressiva que a causada por *P. cinnamomi*. Verificou-se que *Pythium sterilium* é um novo patogénio que afeta as raízes de espécies de *Quercus*, havendo ainda poucos registos do seu isolamento da rizosfera de carvalhos em declínio da região centro de Espanha.

(9)

- Analisaram-se as causas da podridão radicular de plantas com 1 ano (imediatamente antes do transplante) de *Q. ilex* subsp. *ballota* em dois viveiros do sul de Espanha. Foram isolados os agentes patogénicos *P. cinnamomi*, *P. cryptogea* e *P. drechsleri* em plantas que apresentavam sintomatologia de desfoliação e necroses radiculares. Os ensaios de inoculação destas espécies de *Phytophthora*, em azinheira e sobreiro, mostraram maior virulência de *P. cinnamomi* em todas as plantas, e maior suscetibilidade da azinheira que do sobreiro ao patogénio. Este foi o primeiro estudo, em viveiros, sobre o efeito de *P. cinnamomi*, *P. cryptogea* e *P. drechsleri* em *Q. ilex* subsp. *ballota*.

(10)

- Num estudo realizado, entre 1998 e 1999, em azinheiras em declínio na província de Huelva (sudoeste de Espanha), em que se fizeram testes de inoculação em condições controladas, verificou-se a patogenicidade dos isolados de *P. cinnamomi* em plantas jovens de azinheira e sobreiro. A desfoliação e mortalidade apresentadas por estes carvalhos Mediterrânicos (sintomatologia de declínio) foi atribuída ao agente primário *P. cinnamomi*, embora outros fatores como a alternância de períodos secos e húmidos possam ser importantes.

(11)

- Isolaram-se 34 espécies de fungos em sobreiros da Catalunha (nordeste de Espanha) entre 1992 e 1995 e testou-se a sua patogenicidade (inoculações em troncos, folhas e raízes). Verificou-se que 11 destas espécies eram patogénicas ao nível do tronco (*Biscogniauxia mediterranea*, *Botryosphaeria stevensii*, *Diatrype* ci. *stigma*, *Endothia gyrosa*, *Fusarium solani*, *Graphium* sp., *Ophiostoma quercus*, *Phomopsis* sp., *Phytophthora cinnamomi*, *Sporendocladia bactrospora* e uma espécie não identificada pertencente à classe Coelomycete) e 3 espécies eram patogénicas ao nível das folhas (*Dendrophoma myriadea*, *Lembosia quercina* e *Phomopsis quercella*).¸Não se detetou patogenicidade ao nível das raízes. Ao nível do tronco, apenas *P. cinnamomi*, *B. stevensii* e *Phomopsis* sp. provocaram mortalidade, além de cancros e necroses vasculares. O stress hídrico agravou a sintomatologia causada por *B. mediterranea* e *Phomopsis* sp. mas não influenciou significativamente a patogenicidade de *B. stevensii*, espécie mais virulenta de todas as testadas.

(12)

- A partir de amostragens de solo da rizosfera de azinheiras com sintomatologia de declínio, (Extremadura, Espanha), realizaram-se ensaios em ambiente controlado com solo esterilizado e não esterilizado. As plantas jovens que cresceram em ambiente não esterilizado apresentaram redução de crescimento vegetativo e mortalidade. *P. cinnamomi* foi a espécie mais consistentemente isolada das raízes, quando comparada com várias espécies de *Pythium* e *Fusarium oxysporum*, o que sugere que seja um agente de declínio.

(13)

Isolamento de oomicetas e fungos em espécies arbustivas e em espécies de *Quercus* em Itália

- *Phytophthora cinnamomi* foi o principal agente isolado em raízes de *Q. ilex* (sul de Itália). Todos os isolados recolhidos revelaram bastante agressividade em plantas jovens de diferentes espécies de *Quercus* (*Q. suber*, *Q. ilex*, *Q. coccifera*). Pc foi considerado o principal agente de declínio das azinheiras na região.

(1)

- Em amostras de solo e da rizosfera de *Q. ilex* e de arbustos (*Asparagus albus*, *Cistus* sp., *Juniperus phoenicea*, *J. oxycedrus*, *Pistacia lentiscus* e *Rhamnus alaternos*) da vegetação do maquis Mediterrânico com sintomas de declínio (nordeste da Sardenha, Itália) foram identificados além de *P. cinnamomi*, *P. asparagi*, *P. bilorbang*, *P. cryptogea*, *P. gonapodyides*, *P. melonis*, *P. syringae*, *P. crassamura* sp. nov. e *P. ornamentata* sp. nov. Os testes de patogenicidade confirmaram o seu possível envolvimento no declínio no arquipélago de La Maddalena.

(3)

- Na Ilha Caprera (Itália) foram isoladas quatro espécies de fungos pertencentes à família Botryosphaeriaceae (*Botryosphaeria dothidea*, *Diplodia corticola*, *D. seriata* e *Neofusicoccum parvum*), a partir de cancros no tronco e ramos de azinheira, e três espécies de *Phytophthora* (*P. cinnamomi*, *P. cryptogea* e *P. gonapodyides*) a partir de raízes finas e amostras de solo da rizosfera. Verificou-se, em ensaios de patogenicidade, que *Diplodia corticola* era a espécie mais agressiva. O declínio na região foi atribuído ao efeito combinado dos agentes *D. corticola* e *P. cinnamomi*.

(5)

II – Área Temática "Fatores Bióticos – Hospedeiro"

- Analisou-se a suscetibilidade de diferentes espécies de *Quercus* e *Pinus* a *Phytophthora ramorum* e *P. cinnamomi*. Verificou-se moderada suscetibilidade de *Q. ilex* a *P. ramorum*. Observou-se variação genética entre populações na suscetibilidade do hospedeiro e, nalgumas espécies de *Quercus*, uma considerável variação sazonal na suscetibilidade do hospedeiro.

(30)

- A sustentabilidade do montado de sobro tem sido ameaçada pelo declínio, agravado pela pressão humana e pelas alterações climáticas. Estas perturbações têm provocado alterações no equilíbrio da comunidade microbiana da rizosfera das árvores (microbioma). Neste trabalho é feita uma revisão dos principais fatores bióticos e abióticos envolvidos no declínio e do seu impacto no microbioma da rizosfera. Sugerem-se algumas estratégias de gestão para melhoria da sustentabilidade.

(17)

- Com base numa rede de monitorização de danos causados por patogénicos, e num conjunto de variáveis ambientais (meteorológicas, edáficas, topográficas e de coberto florestal), foram aplicados modelos para previsão da distribuição presente e futura de *P. cinnamomi* na Andaluzia (Espanha) para diferentes cenários de alterações climáticas. Classificaram-se as zonas analisadas de acordo com a presença e probabilidade de ocorrência do patogénio, tendo sido identificadas zonas de intervenção prioritária. Sugerem-se medidas fitossanitárias para cada uma das zonas.

(18)

- Avaliou-se a tolerância de diferentes proveniências de sobreiro a *P. cinnamomi* com o objetivo de selecionar famílias tolerantes para reflorestação. De 2004 a 2014, num ensaio com solos fortemente infetados com *P. cinnamomi*, avaliaram-se 157 famílias de 8 proveniências de Portugal e Espanha. Observou-se elevada mortalidade. Apenas 14 das 157 famílias tiveram taxas de sobrevivência entre 40 e 60% e bom crescimento em altura. Verificou-se também boa resistência de plantas de *Q. faginea* a *P.*

cinnamomi, aspeto a considerar em reflorestações. A mobilização do solo poderá contribuir para melhorar a taxa de sobrevivência e desenvolvimento das plantas.

(19)

- Avaliou-se o efeito da interação de várias espécies de *Phytophthora* (*P. cinnamomi*, *P. gonapodyides* e *P. quercina*) na germinação e sobrevivência de plantas de 16 populações de *Quercus ilex* (subespécies *ballota* e *ilex*). As taxas de germinação das sementes não dependeram da espécie de *Phytophthora* mas das populações e subespécies de *Q. ilex*. Em solos infestados com *espécies de Phytophthora*, a taxa de germinação das sementes foi maior em *Q. ilex* subsp. *ilex* que em *Q. ilex* subsp. *ballota*. A sobrevivência das plantas foi fortemente influenciada pela espécie de *Phytophthora*, nao dependendo da subespécie ou população de *Q. ilex*. Em plantas infetadas primeiro com espécies de *Phytophthora* menos virulentas e só depois com espécies mais virulentas, houve diminuição e atraso na mortalidade. Verificou-se que em solos infetados com espécies de *Phytophthora* as sementes grandes são vantajosas porque proporcionam um rápido crescimento inicial.

(20)

- Apesar dos sintomas causados pela infeção de *P. cinnamomi* em *Quercus suber* e *Q. ilex* serem semelhantes, as taxas de mortalidade diferem. Observaram-se diferenças no crescimento do sistema radicular após infeção, dependentes da espécie arbórea, tamanho inicial da planta e nível do inóculo. Em *Q. ilex* observou-se uma diminuição apreciável no crescimento de novas raízes com o aumento do nível de inóculo. Em *Q. suber* observaram-se raízes mais compridas e menos espessas para níveis moderados de inóculo. Nas duas espécies observou-se um pior estado hídrico das plantas mesmo para baixos níveis de inóculo.

(21)

- Avaliou-se a suscetibilidade de quatro morfotipos de *Quercus ilex* subsp. *ballota* (expansa, macrocarpa, microcarpa e rotundifolia) a *P.* cinnamomi na Andaluzia (Espanha). Observou-se uma resposta muito semelhante dos morfotipos ao nível da sintomatologia radicular (elevado grau de necroses) e diferenças na sintomatologia das folhas (*microcarpa* – muito suscetível; *expansa* – suscetível; *rotundifolia* e *macrocarpa* – moderadamente suscetíveis). O híbrido natural *Q. ilex* subsp. *ballota* – *Q. faginea* exibiu baixa suscetibilidade a nível radicular e foliar. *Q. faginea* poderá ser uma fonte de resistência a considerar em programas de melhoramento.

(28)

- Analisou-se a sobrevivência de plantas de 1 ano de azinheira durante dois anos, em solos naturalmente contaminados por *P. cinnamomi* (sudoeste de Espanha). Neste período praticamente não se detetou mortalidade no outono e no inverno. A mortalidade aumentou na primavera (sobretudo no primeiro ano) e, mais acentuadamente, no verão. Cerca de 20% da mortalidade ocorrida durante os dois anos foi devida a *P. cinnamomi*. O padrão de mortalidade parece refletir a distribuição agregada do inóculo no solo e evidencia maior ocorrência próximo de árvores adultas infetadas.

(32)

- Num estudo em ambiente controlado, analisou-se a emergência e sobrevivência de bolotas pré-germinadas de sobreiro e azinheira em solos naturalmente infetados com *P. cinnamomi*, e nos mesmos solos esterilizados, de duas *dehesas* com diferentes características ecológicas. O patogénio foi isolado das radículas das bolotas que não emergiram e das plantas mortas dos solos não esterilizados. As plantas de azinheira revelaram maior suscetibilidade ao patogénio que as de sobreiro. A mortalidade em azinheira foi de 100% em solos não esterilizados, e não dependeu da proveniência da semente ou da origem do solo. A mortalidade em sobreiro dependeu da origem e tratamento do solo (esterilizado ou não esterilizado), das interações origem da bolota x origem do solo e origem do solo x tratamento do solo. A elevada suscetibilidade das plantas de azinheira e de sobreiro a *P. cinnamomi* é um fator limitante para a regeneração natural e reflorestação por sementeira direta.

(33)

- Analisaram-se, durante dois anos, os efeitos de *P. cinnamomi* nas relações hídricas, acumulação de biomassa, nutrição mineral e vulnerabilidade ao défice hídrico de plantas jovens de *Quercus ilex*, *Q. rubra*, *Q. robur* e comparou-se com *Castanea sativa* (suscetível) e com um híbrido resistente (*Castanea sativa* x *C. crenata*). Os resultados mostraram maior suscetibilidade da azinheira a *P. cinnamomi*, com maior perda de raízes (67%) e mortalidade de 10% em plantas inoculadas bem irrigadas. A perda de raízes foi associada a um decréscimo no potencial hídrico foliar de madrugada, a reduções na condutância estomática (61%), na biomassa aérea (55%) e nos teores de azoto (N) e fósforo (P) nas folhas (relativamente ao controlo).

(34)

- Em plantas com 1 ano de idade de *Q. suber* e *Q. ilex* analisou-se, numa primeira fase, o efeito conjugado da infeção por *P. cinnamomi* com o de encharcamento e, numa segunda fase, em plantas de 2 anos de *Q. suber*, *Q. ilex* e *Q. rubra*, o efeito da infeção seguida de 2 ciclos de seca - recuperação. Os resultados mostraram que *Q. ilex* foi a espécie com maior suscetibilidade à infeção e *Q. rubra* a de menor suscetibilidade. As infeções radiculares causadas por *P. cinnamomi* foram mais severas em condições de encharcamento que em situação de défice hídrico. O principal efeito da infeção nas relações hídricas foi um decréscimo da condutância estomática. A inoculação causou um decréscimo no potencial hídrico foliar de madrugada em *Q. ilex* e nas plantas de *Q. suber* que tiveram perdas severas de raízes. A infeção por *P. cinnamomi* alterou a relação condutância estomática - potencial hídrico foliar de madrugada. O encharcamento e a infeção atuaram sinergisticamente nas relações hídricas em *Q. ilex*, não tendo havido agravamento da doença com a imposição de stress hídrico após a inoculação com o patogénio.

(35)

- *Phytophthora cinnamomi* foi isolado de amostras do solo e raízes de *Quercus suber* e *Q. ilex* com doença, em zonas de declínio. Admite-se que a infeção com o patogénio possa ser um fator fundamental de declínio, interagindo com o stress hídrico e outros fatores locais que podem tornar as árvores vulneráveis ao ataque de insetos e fungos.

(36)

Mecanismos de defesa do hospedeiro

- Os taninos são compostos produzidos pelas plantas como defesa contra stresses de origem biótica (ex.: outros microrganismos) e abiótica (ex.: falta de água). Analisou-se a possível regulação da síntese de taninos pelo stress biótico em *Q. ilex*. Os resultados mostraram que a síntese de taninos em plantas jovens de azinheira é induzida por desfolha mecânica, enquanto que a infeção por *P. cinnamomi* interfere na regulação deste processo, aumentando a suscetibilidade das plantas à herbivoria e agravando o impacto do stress biótico.

(16)

- A maioria das espécies de *Phytophthora* produz elicitinas, moléculas que podem estimular reações de defesa do hospedeiro contra a invasão por agentes patogénicos. Analisou-se a indução, por cinamominas, de mecanismos de defesa contra o patogénio em *Quercus ilex* subsp. *rotundifolia* e *Q. suber*. O pré-tratamento com alfa-cinamomina confinou a colonização pelas hifas do patogénio nas duas espécies. Em *Q. suber* observou-se um decréscimo considerável da infeção 24 horas após tratamento com alfa-cinamomina e beta-cinamomina.

(23)

- Foi estudado o efeito de diferentes elicitinas (proteínas), ao nível da célula e da fisiologia da planta, em sobreiros infetados por *P. cinnamomi*. Verificou-se que a criptogeína (elicitina), ou a sua interação com *P. cinnamomi*, induziu o aumento da síntese de lípidos nas folhas, o que pode contribuir para preservar a estabilidade das membranas. Foi analisada a progressão do patogénio no tecido radicular, os seus efeitos na composição em ácidos gordos totais das raízes e folhas e foram efetuadas medições de parâmetros fotossintéticos. Nas raízes tratadas com elicitina, dois dias após a inoculação, observou-se perda de viabilidade do patogénio com confinamento aos espaços intercelulares do parênquima cortical, não atingindo o cilindro vascular. *P. cinnamomi* originou diminuição da fotossíntese líquida, condutância estomática, fluorescência da clorofila e carotenóides. As elicitinas parecem promover no sobreiro respostas de defesa contra a infeção por *P. cinnamomi*.

(27), (29), (31)

Histologia

- A análise histológica de tecidos radiculares de azinheira infetados por *P. cinnamomi* mostrou haver, mais frequentemente, presença de hifas nos tecidos do xilema de raízes secundárias do que de raízes primárias, embora nos dois tipos de raízes o floema seja o trajeto mais importante para colonização.

(24)

- Estudos histopatológicos da infeção e colonização por *P. cinnamomi* em raízes finas de azinheira permitiram descrever a interação entre hospedeiro e agente patogénico. Nos estágios iniciais da infeção, *P. cinnamomi* coloniza o apoplasto e penetra nas células corticais. Ao atingir as células dos tecidos parenquimatosos do cilindro central, o patogénio desenvolve diferentes estruturas reprodutivas e de sobrevivência e, em seguida, expande-se através do sistema vascular da raiz. Foram identificadas algumas reações do hospedeiro, tais como espessamento da parede celular, acumulação de compostos fenólicos na lamela média dos tecidos esclerenquimatosos e secreções mucilaginosas bloqueando células vasculares. A rápida expansão do patogénio impede, em grande parte, o efeito das respostas do hospedeiro.

(25)

Micorrizas

- Avaliou-se a abundância e variabilidade sazonal de ectomicorrizas em áreas de azinheira (oeste de Espanha), bem como as infeções radiculares causadas por *P. cinnamomi.* Verificou-se que *Cenococcum geophilum*, *Tomentella* spp. e *Russula* spp. foram os morfotipos de micorrizas mais abundantes. As variações sazonais de micorrizas foram maiores para *Tomentella* spp. do que para *Russula* spp. e *Cenococcum geophilum*, mas não foram influenciadas pela topografia, estado de declínio das árvores ou infeções radiculares causadas por *P. cinnamomi*. Verificou-se que a proporção entre terminações de raízes viáveis e não viáveis para as micorrizas é tendencialmente menor em árvores em declínio.

(22)

- O declínio de *Q. ilex* e a presença de *P. cinnamomi* podem estar associados a variações na abundância de fungos ectomicorrízicos. As características do solo podem influenciar a relação do patogénio com os fungos ectomicorrízicos. Analisou-se esta associação em 96 povoamentos em zonas com e sem declínio (sudoeste de Espanha) e verificou-se que o processo de micorrização ocorre com maior intensidade em árvores saudáveis que em árvores em declínio. As árvores situadas nas margens de cursos de água apresentaram menor abundância de raízes com ectomicorrizas em solos de textura fina do que de textura grosseira. A abundância de ectomicorrizas nas raízes é proporcional à espessura do horizonte Ah*** , independentemente do estado sanitário das árvores. As terminações das raízes não viáveis constituem zonas vulneráveis de entrada do patogénio na árvore.

(26)

III – Área Temática "Fatores Abióticos"

Os fatores abióticos influenciam o estado fisiológico e sanitário das árvores e da vegetação do sobcoberto de montados e *dehesas*

- *Phytophthora cinnamomi* é um patogénio agressivo que destrói o sistema radicular de *Quercus su*ber e *Q. ilex* na região Mediterrânica. Desenvolve o seu ciclo de vida no solo, necessitando de condições de temperatura e humidade específicas para infetar as raízes. Em conjunto com a seca é um dos fatores de declínio dos carvalhos Mediterrânicos. O aumento de temperatura, devido às alterações climáticas, pode potenciar a atividade do patogénio em certas zonas (oeste do Mediterrâneo, noroeste da Europa), mas não noutras com invernos frios (centro e este da Europa).

(45)

- Em montados de sobreiro e azinheira, de diferentes regiões e locais de Portugal, foi possível isolar *P. cinnamomi* de raízes e de amostras de solo da rizosfera. Verificou-se

*** Horizonte Ah: horizonte mineral rico com percentagem de matéria orgânica inferior a 30% (FAO, 2006).

maior ocorrência de *P. cinnamomi* em encostas viradas a sul, vales e solos delgados (Leptossolos e Luvissolos). Os solos com baixa fertilidade e baixos níveis de nutrientes minerais, particularmente fósforo, parecem favorecer a infeção. No sobcoberto as espécies infetadas foram sobretudo plantas arbustivas das famílias Ericaceae, Cistaceae e Leguminosae.

(42)

- Avaliou-se a variação sazonal da suscetibilidade do sobreiro aos agentes patogénicos *P. cinnamomi* e *Botryosphaeria stevensii*, em função da variação nos fatores ambientais e estado hídrico das plantas. Os sintomas de infeção foram detetados principalmente em plantas jovens inoculadas entre a primavera e o outono. Valores médios da temperatura mínima diária entre 5 e 12° C inibiram o desenvolvimento da lesão provocada por *P. cinnamomi.* Em *B. stevensii* a diminuição na expansão do cancro foi menos pronunciada. Em raminhos inoculados mensalmente com *B. stevensii* observou-se uma relação linear negativa entre os valores de teor relativo em água da planta (81-91%) e a extensão do cancro. As lesões provocadas por *P. cinnamomi* não estavam relacionadas com alterações sazonais no teor de água.

(44)

Em ambiente natural os fatores abióticos conjugam-se frequentemente com fatores bióticos, nomeadamente com *P. cinnamomi*

- Avaliou-se o efeito conjugado do stress hídrico e da infeção por *P. cinnamomi* na fotossíntese, alocação de biomassa e sistema radicular de plantas jovens de azinheira. Observou-se uma redução significativa na biomassa radicular devido à podridão radicular causada pela infeção. Na ausência de outros fatores de stress, as plantas jovens reduziram o aumento de biomassa e realocaram recursos.

(37)

- Analisaram-se as condicionantes da variabilidade espacial (larga escala) na distribuição dos microrganismos do solo (*Phytophthora cinnamomi*) na Península Ibérica e procurou-se modelar essa distribuição em função de variáveis abióticas (clima e solo), bióticas (distribuição do hospedeiro principal e coberto vegetal baseado no NDVI) e influência do homem. Os resultados mostraram que a distribuição atual de *P. cinnamomi* na Península Ibérica é influenciada principalmente pela textura fina do solo e clima, uso do solo e presença do hospedeiro (árvore) principal. Parece haver potencial para expansão do patogénio para nordeste e sudeste de Espanha e centro de Portugal.

(38)

- Analisou-se a influência da temperatura na germinação de bolotas de *Quercus ilex*, em solos infetados com *P. cinnamomi*, *P. gonapodyides*, *P. quercina* e *P. psychrophila*. Os danos causados na radícula e a mortalidade variaram com a espécie de *Phytophthora* presente no solo, o que indica que é a interação espécie de *Phytophthora* x temperatura que determina a germinação das bolotas de *Q. ilex*.

(39)

- O stress hídrico aumentou a suscetibilidade de plantas jovens de azinheira à infeção por *P. cinnamomi*. Após a infeção observou-se um aumento nas taxas de mortalidade. Em solos infetados com *P. cinnamomi*, o encharcamento seguido de stress hídrico causou 100% de mortalidade.

(40)

- Analisou-se o efeito do estado hídrico das árvores, dinâmica da água do solo e abundância de raízes finas em *dehesas* de *Quercus ilex* (oeste de Espanha), com solo infetado e não infetado por *P. cinnamomi*. Os resultados revelaram reduções significativas de potencial hídrico foliar e de condutância estomática em árvores em declínio. Os períodos de encharcamento e o teor de água do solo não foram só por si suficientes para explicar o estado sanitário das árvores. A conjugação da infeção por *P. cinnamomi,* com destruição de raízes finas, com períodos de solo saturado (favoráveis ao patogénio mas desfavoráveis para as árvores) e diferenças locais na capacidade de retenção de água do solo explicam os sintomas de declínio das árvores e o seu estado hídrico, sendo fatores que muito provavelmente determinam a sobrevivência das árvores.

(41)

- As respostas de plantas jovens de *Quercus ilex* e *Q. cerris* a fatores de stress, como o stress hídrico e o stress biótico causado por infeção por *P. cinnamomi*, parecem envolver a presença de determinadas proteínas (*dehydrina-like*).

(43)

IV – Área Temática "Biologia Molecular/Bioquímica/Fisiologia"

- Para conhecer o impacto de alterações na riqueza e diversidade de espécies do micobioma do solo no declínio de *dehesas*, utilizou-se o método de *metabarcoding*. Com base no DNA total das amostras de solo verificou-se que a diversidade e composição das comunidades de fungos e oomicetas estava relacionada com a severidade de declínio. Existia uma importante comunidade de micorrizas e fungos patogénicos: *Phytophthora cinnamomi* e *Pythium spiculum* não estavam entre os oomicetas mais abundantes, nem estavam diretamente relacionados com os níveis de desfoliação; a reduzida presença de espécies de *Phytophthora* estava fortemente correlacionada com espécies do género *Trichoderma*. O estudo do microbioma com base no *metabarcoding* é uma ferramente valiosa para desenvolver estratégias de biocontrolo. Os resultados deste estudo mostram a importância de se analisarem as interações entre diferentes componentes do microbioma do solo e de se avaliar o efeito da aplicação de espécies de *Trichoderma* no controlo de oomicetas, e da micorrização na melhoria do estado fitossanitário das árvores.

(46)

- As técnicas de micropropagação, *i.e.*, de multiplicação *in vitro*, permitem quer a propagação de espécies lenhosas, quer a sua conservação no curto e longo prazo. A partir de plântulas de azinheira com 3 meses de idade, provenientes de bolotas germinadas em condições controladas de humidade e temperatura, foi possível micropropagar a azinheira apesar de ser uma planta lenhosa e de difícil manutenção *in vitro*. No entanto, o sucesso da cultura *in vitro* desta espécie depende do genótipo, quer na fase de estabelecimento, quer na de enraizamento. A metodologia utilizada poderá ser usada na produção em larga escala e na conservação da azinheira. É necessário otimizar as condições de micropropagação para obtenção de maior número de genótipos estabelecidos e enraizados.

(47)

- Analisaram-se os genes (transcriptómica e genómica) de *Phlomis purpurea* (marioila), espécie que produz compostos bioativos envolvidos na atividade anti-oomiceta. Esta informação permite compreender os mecanismos de defesa subjacentes à interação patogénio *Phytophthora cinnamomi*/planta.

(48)

- *Phytophthora cinnamoni* tem sido associada ao declínio das quercíneas das *dehesas* das regiões oeste, centro e sul de Espanha. Na região este e em solos calcários, o declínio foi associado às espécies *P. quercina* e *P. psychrophilla*. A análise de solos e raízes por pirosequenciação e o método de *metabarcoding* mostraram uma diversidade de espécies de *Phytophthora* e o predomínio de uma espécie inédita com nome informal "*Phytophthora taxon ballota*". Foi proposto um novo protocolo de *RT-PCR* (*real-time PCR*) baseado nos resultados da pirosequenciação, para a deteção das espécies de *Phytophthor*a associadas ao declínio nas *dehesas* da zona este de Espanha.

(49)

- Isolaram-se quatro compostos do metabolismo secundário de *Diplodia corticola* (fungo fitopatogénico associado ao declínio do sobreiro) e estudou-se a sua atividade em plantas, noutros fungos, em oomicetas e em animais. Um destes compostos revelou atividade fitotóxica em folhas de *Quercus afares*, *Quercus suber*, *Quercus ilex* e *Celtis australis* na concentração de 1 mg/mL (lesões necróticas) e inibiu o crescimento do micélio de *Athelia rolfsii*, *Lasiodiplodia mediterranea* e *Phytophthora cinnamomi.*

(50)

- A espectroscopia FT-IR (*Fourier-transformed infrared*) em combinação com a quimiometria permite revelar os padrões do metabolismo de raízes de sobreiro e micélio de *P. cinnamomi* durante as primeiras horas da interação. Os resultados revelaram alterações precoces nos padrões metabólicos do hospedeiro ao interagir com o patogénio e alterações (embora em menor grau) nos padrões metabólicos de *P. cinnamomi* quando expostos a exsudados da raiz do sobreiro.

(51)

- A análise quantitativa de genes diferencialmente expressos recorrendo à técnica de *RT-PCR* permitiu conhecer o perfil genético de alguns genes importantes na resposta de *Q. suber* à infeção por *P. cinnamomi*. O conhecimento das interações moleculares que envolvem o sobreiro e *P. cinnamomi* pode ser útil para compreender os mecanismos subjacentes de resistência do sobreiro a estes agentes fitopatogénicos.

(52)

- Num estudo sobre a anatomia e desenvolvimento da endoderme e felema em sobreiro verificou-se que os componentes químicos da suberina estão dispostos na parede celular de acordo com o papel fisiológico ou estado de maturação de um dado tecido. As zonas da raiz em que há deposição de suberina parecem constituir barreiras à entrada de agentes patogénicos como *P. cinnamomi*.

(53)

- Foram analisadas as respostas fisiológicas (estado hídrico e parâmetros fotossintéticos) e os perfis proteicos foliares de plantas jovens de azinheira inoculadas e não inoculadas com *P. cinnamomi*. Nas plantas inoculadas com o patogénio observou-se uma redução nos valores do teor de água, fluorescência da clorofila, condutância estomática e trocas gasosas, comparativamente às plantas não inoculadas. As plantas inoculadas tiveram também um decréscimo do teor proteico, apesar de aumento no teor de proteínas associadas à biossíntese do amido e da glicólise. Estas alterações nos teores proteicos estão de acordo com os parâmetros fisiológicos estimados e com as respostas observadas em plantas submetidas a stress hídrico.

(54)

- Analisou-se a expressão diferencial de quatro genes de elicitina de *Phytophthora cinnamomi* recorrendo à técnica de *RT-PCR*. Em culturas *in vitro*, o gene alfa-cinamomina demonstrou o maior nível de expressão e o gene beta-cinamomina foi o mais induzível. Verificou-se também que a transcrição das elicitinas esteve ativa durante o período de crescimento ativo do patogénio no processo de infeção de raízes de sobreiro e que, à medida que a colonização do hospedeiro progrediu, o nível de expressão da beta-cinamomina diminuiu, enquanto o de alfa-cinamomina aumentou, sugerindo que as elicitinas estão relacionadas, direta ou indiretamente, com o mecanismo da patogénese.

(55)

- Foi identificado em sobreiro um gene (que codifica para a enzima QsCAD1) com elevada expressão em raízes infetadas com *P. cinnamomi*. Os resultados sugerem que esta enzima está potencialmente envolvida na desativação de toxinas produzidas por agentes fitopatogénicos, nomeadamente *P. cinnamomi*, constituindo uma resposta de defesa à infeção.

(56)

V – Área Temática "Prevenção e Controlo"

A prevenção e controlo de *Phytophthora cinnamomi* pode envolver a aplicação de fungicidas, adubos químicos, tratamentos à base de extratos de plantas e a aplicação de corretivos orgânicos (estrumes e chorumes)

Aplicação de fungicidas

- Em ensaios *in vitro* com plantas de *Quercus suber* e *Q. ilex* comparou-se o efeito do fosfito de potássio e do fosetil de alumínio em *P. cinnamomi*, usando o metalaxil (fungicida de reconhecida eficácia contra *P. cinnamomi*) como referência. Os resultados mostraram que o fosetil de alumínio é uma alternativa ao fosfito de potássio, sendo eficaz no controlo da doença provocada por espécies de *Phytophthora*.

(57)

- Analisou-se o efeito da aplicação de fosfonato de potássio, por injeção, em azinheira (sudoeste de Espanha) em condições de presença de *P. cinnamomi* e de stress hídrico. Avaliaram-se as características do solo, mediu-se o teor de água do solo e a profundidade do lençol freático em locais com diferentes incidências de declínio. Após cinco anos de tratamentos não houve diferenças significativas entre dferentes modalidades de tratamento e as árvores não injetadas, o que sugere que, nas condições experimentais testadas, o declínio teria sido devido a stress hídrico. A eficácia do tratamento com fosfonato de potássio em áreas com reduzida humidade no solo e baixa contaminação foi reduzida.

(63)

- Testou-se a aplicação, por injeção, de fosfonato de potássio, quinosol e carbendazime em sobreiro e azinheira (Extremadura, Espanha). As árvores injetadas com fosfonato de potássio mostraram uma melhoria significativa no crescimento vegetativo dois anos após o tratamento, tendo também evidenciado alguma recuperação dos sintomas de declínio durante o terceiro ano. Tratando-se de uma substância ativa com efeito fungicida, os resultados deste trabalho corroboram indiretamente a implicação de *P. cinnamomi* no declínio das zonas estudadas.

(65)

Aplicação de adubos químicos

- Testou-se a suscetibilidade de plantas jovens de *Q. ilex* à infeção por *P. cinnamomi* (sudoeste de Espanha) usando uma solução nutritiva standard (com cálcio Ca^{2+} e potássio K^{+}), uma solução deficiente em K^{+} e uma solução deficiente em Ca^{2+}. As azinheiras submetidas a deficiência em K^{+} apresentaram valores elevados de Ca^{2+} e foram tolerantes à doença. As submetidas a deficiência em Ca^{2+} não apresentaram níveis elevados de K^{+} e tiveram menor desenvolvimento radicular. A adição de K^{+} não conferiu maior tolerância à doença. Os resultados mostram que as fertilizações cálcicas podem conferir maior resistência à doença.

(61)

- Tendo sido observada fraca incidência de doença radicular provocada por *P. cinnamomi* em solos com teores médios de Ca^{2+}, analisou-se a capacidade de fertilizantes cálcicos induzirem a supressão de *P. cinnamomi* do solo. Ensaios *in vitro*, em que foram testados diferentes fertilizantes cálcicos, mostraram inibição significativa de esporângios, clamidósporos e zoósporos de *P. cinnamomi*. Ensaios em estufa, utilizando solos infetados artificialmente com *P. cinnamomi*, mostraram uma redução significativa na severidade dos sintomas foliares e radiculares com fertilizações cálcicas. Dum modo geral os trabalhos realizados revelaram que, em plantas jovens de azinheira, as fertilizações cálcicas podem reduzir os efeitos da doença provocada por *P. cinnamomi*, devido principalmente à inibição da produção de esporos.

(62)

Aplicação de extratos de plantas com efeito supressivo

- Alguns tecidos de plantas, nomeadamente de espécies da família Brassicacea, são ricos em glucosinolatos, compostos com interesse em programas de luta contra patogénios do solo. A hidrólise destes compostos origina isotiocianatos aos quais se atribui a toxicidade para microrganismos do solo, nomeadamente *P. cinnamomi*. Avaliou-se em condições controladas a eficácia de 14 espécies da família Brassicaceae com atividade de biofumigação. As espécies ricas em sinigrina suprimiram em 100% o crescimento do micélio de *P. cinnamomi* enquanto as ricas noutros glucosinolatos quase não tiveram efeito supressivo. Em condições de campo, testou-se o efeito de três espécies com diferentes glucosinolatos: *Brassica juncea*, *Eruca vesicaria* e *Lepidium sativum*. *Brassica juncea* e *Eruca vesicaria* reduziram a viabilidade dos clamidósporos mas *L. Sativu*m não teve qualquer efeito. Apesar de reduzir o inóculo, a biofumigação com *B. juncea* não evitou a infeção em tremocilha (*Lupinus luteus*, hospedeiro muito suscetível). A biofumigação por si só não se mostrou eficaz na supressão substancial de inóculo de *P. cinnamomi*.

(58)

- Analisou-se, *in vitro,* em solos infetados em laboratório e, *in planta*, a forma como a biofumigação com *Brassica juncea*, *Brassica carinata e Brassica napus* afeta fases críticas do ciclo de vida de *P. cinnamomi*. *Brassica juncea* e *Brassica carinata* inibiram o crescimento do micélio, reduziram a produção de esporângios e inibiram a viabilidade dos clamidósporos no solo, embora só *B. carinata* tenha reduzido os sintomas da doença. As espécies de Brassicacea ricas em sinigrina são mais indicadas no controlo da doença radicular provocada por *P. cinnamomi.*

(59)

- *Phlomis purpurea* (marioila; Lamiaceae) é uma planta não hospedeira de *P. cinnamomi* frequente nos montados de sobro e azinho em Portugal. Avaliou-se, *in vitro*, o efeito dos seus extratos no crescimento do micélio, produção de esporângios, libertação de zoósporos e germinação, produção e viabilidade de clamidósporos. Avaliou-se também, *in planta*, a proteção de sobreiro contra a infeção pelo patogénio. Os resultados sugerem que *P. purpurea* pode reduzir a disseminação da doença e que os seus extratos podem ser importantes no controlo de *P. cinnamomi*.

(60)

Aplicação de extratos de plantas com efeito supressivo e corretivos orgânicos (estrumes e chorumes)

- Avaliou-se o efeito de biofumigação do solo com *Brassica carinata*, *Brassica juncea*, *Cistus albus*, *Cistus ladanifer*, *Diplotaxis sp*., e *Phlomis purpurea* e também o efeito da incorporação no solo de estrumes frescos e compostados (gado bovino, suíno, ovino e de aviário) no crescimento do micélio de *P. cinnamomi* e na redução de inóculo do solo contaminado. Os resultados mostraram que as espécies de Brassicaceae, os estrumes de aviário e os chorumes de suínos foram os mais eficazes na redução do inóculo.

(64)

VI – Área Temática "Ecologia"

- Para prever o impacto das alterações climáticas no declínio do sobreiro avaliaram-se 10 locais e duas populações/local ao longo de um gradiente de aridez. Relacionaram-se parâmetros como a latitude, aridez, presença de agentes patogénicos (*Phytophthora cinnamomi*), densidade dos povoamentos e tamanho das árvores, com o tamanho das sementes, estrutura demográfica, dominância do banco de sementes do solo, desfoliação e mortalidade. Observou-se um aumento do peso da semente com o decréscimo da latitude; menor regeneração natural no limite sul da área de distribuição com maior dominância de sobreiros envelhecidos. A estrutura demográfica foi influenciada pela latitude, precipitação e abundância do patogénio. Os resultados obtidos permitem a deteção precoce dos riscos e tendências de declínio e sugerem deslocação da espécie devido a falhas na regeneração no limite sul e uma possível expansão do sobreiro das zonas a norte.

(66)

- É feita uma revisão do impacto de *P. cinnamom*i nos ecossistemas florestais, a nível internacional. Sugere-se a adoção de 3 estratégias principais para melhorar a resiliência desses ecossistemas: melhoria das condições locais para reduzir riscos de doença; melhoramento genético das espécies mais susceptíveis a *P. cinnamom*i; implementação de medidas de prevenção para evitar a disseminação do patogénio, em particular a partir de viveiros.

(67)

- Foi feita uma análise da variação espacial (ao nível da árvore, local, paisagem) do declínio em sobreiro e da forma como as árvores ajustam a sua fisiologia (eficiência de uso de água (EUA) e crescimento secundário). Ao nível da árvore, não se observaram diferenças na EUA ou crescimento secundário entre árvores sãs e com desfoliação. À escala local e da paisagem, observou-se uma maior EUA induzida por solos delgados e elevada abundância de patogénio. Em condições de seca do solo, e na presença de agentes patogénicos radiculares, o sobreiro pode aumentar a eficiência do uso da água, embora essa resposta possa não ser suficiente para as árvores sobreviverem ao stress fisiológico provocado pelo patogénio.

(68)

- Os *feedbacks* planta-solo podem alterar a abundância das espécies, a sua coexistência e sucessão nas comunidades de plantas. Analisaram-se esses *feedbacks* em dois tipos de povoamentos de *Q. suber* (Espanha) com densidades diferentes. Nos locais com presença de *P. cinnamomi* poderá assistir-se no futuro à perda de dominância de *Q. suber*, sendo favorecidas espécies coexistentes não suscetíveis ao declínio.

(69)

VII – Área Temática "Solo"

- A mortalidade das árvores induzida por *P. cinnamomi*, conjugada com o efeito do aquecimento global (aumento de temperatura e seca), pode conduzir a alterações nos ciclos biogeoquímicos dos solos em áreas ocupadas com *Q. suber*. É expectável que as taxas de respiração do solo e a disponibilidade de nutrientes possam ser afetadas indiretamente pela mortalidade por *P. cinnamomi,* devido à substituição de espécies.

(70, 72)

- Em povoamentos mistos de carvalhos (sul de Espanha), em declínio devido à contaminação por *P. cinnamomi*, analisaram-se diversos fatores que podem promover alterações na fauna do solo à escala local. Avaliaram-se as correlações entre a abundância da fauna do solo e a disponibilidade de luz, caracterísiticas das árvores e folhada, e abundância de *P. cinnamomi*. Observou-se, de acordo com o seu nível trófico, uma correlação positiva entre as abundâncias de predadores e detritívoros; uma correlação positiva entre a abundância de detritívoros e o agente patogénico; uma correlação negativa entre a abundância de detritívoros e a disponibilidade de luz e a desfolha. Embora cada grupo de organismos do solo exiba diferentes condições ótimas de crescimento observou-se um papel predominante da disponibilidade de luz na sua abundância.

(71)

- As características do solo (profundidade, espessura do horizonte Ah, textura, pH, potencial redox, densidade, concentrações $N\text{-}NH_4^+$ e $N\text{-}NO_3^-$) afetam a vitalidade das árvores e a infeção causada por *Phytophthora cinnamomi*. Apesar dos solos de textura fina e horizontes Ah espessos serem mais favoráveis ao vigor e vitalidade das árvores em clima mediterrânico, podem facilitar a infeção das raízes das azinheiras por *P. cinnamomi* (maior ocorrência de plantas infetadas). Estas características do solo estão associadas a níveis elevados de humidade do solo que aumentam o inóculo do patogénio e favorecem as infeções radiculares. Em 96 montados de azinho (oeste de Espanha) com sintomas de declínio, o pastoreio não pareceu estar diretamente associado ao estado sanitário das árvores ou à doença causada por *P. cinnamomi*.

(73)

- A abundância de agentes patogénicos que desenvolvem o seu ciclo de vida no solo (*Phytophthora cinnamomi*, *Pythium spiculum* e *Pythium* spp.) foi analisada em função de características do solo e das árvores e arbustos presentes nos povoamentos de *Q. suber* estudados (com *Q. canariensis* e *Olea europaea*). A abundância do patogénio no solo apresentou um padrão não aletório influenciado, quer por fatores abióticos (textura do solo - menor abundância em solos arenosos), quer bióticos (maior abundância de *Phytophthora cinnamomi* e *Pythium* spp., sob as árvores em declínio com graus de desfoliação superiores a 50%; menor abundância sob *O. europaea* que no solo circundante e maior abundância sob *Q. canariensis* – atua como reservatório sem mostrar sintomas). A abundância de *Phytophthora cinnamomi* no solo reduziu a emergência e sobrevivência das plantas jovens em povoamentos abertos, mas não em todos os locais e espécies. Observou-se, na microescala, um padrão heterogéneo na abundância de alguns patogénios.

(74)

VII – Área Temática "Sobcoberto"

Suscetibilidade de espécies do sobcoberto (herbáceas) a *P. cinnamomi*

- Em ambiente controlado e com inoculações realizadas *in vitro* detetou-se a presença de *P. cinnamomi* em raízes de tremocilha (*Lupinus luteus*; sintomática) e ervilhaca (*Vicia sativa*; assintomática), mas não se detetou em trigo (*Triticum aestivum*) e aveia (*Avena sativa*) (ambas assintomáticas). Verificou-se que a tremocilha estimula a produção de zoósporos de *P. cinnamomi*, o que não acontece com a ervilhaca, o trigo e a aveia.

As culturas de trigo, aveia e ervilhaca não influenciaram a epidemiologia da doença radicular em *Quercus*, mesmo na presença de ervilhaca infetada e sem sintomas.

O trigo, a aveia e a ervilhaca podem ser uma alternativa à cultura de tremocilha em montados/*dehesas* quando *P. cinnamomi* está presente.

(75)

- Em *dehesas* com tremocilha, o isolamento e contagem do nº de colónias de *P. cinnamomi* a partir de amostras de solo mostrou que esta planta é capaz de manter ou aumentar a densidade de inóculo, facilitando a infeção das árvores. Deve-se evitar a cultura de tremocilh*a* em montados e *dehesas*, quer as árvores estejam ou não infetadas por *P. cinnamomi*. Esta leguminosa pode atuar como um reservatório de inóculo ou pode mesmo aumentar os níveis de inóculo do solo, agravando a severidade da doença.

(76)

- Inoculações artificiais com *P. cinnamomi* em quatro cultivares de tremocilha (*Lupinus luteus*) reproduziram os sintomas da doença nas fases de pré e de pós-emergência (confirmação posterior por isolamento em raízes necróticas). Os resultados sugerem que a tremocilha pode constituir um reservatório de inóculo para a infeção de raízes de espécies de *Quercus*. *P. cinnamomi* foi descrito pela primeira vez como patogénio radicular de *Lupinus luteus.*

(77)

Efeito supressivo de espécies do sobcoberto (herbáceas) no inóculo de *P. cinnamomi*

- Estudaram-se compostos bioativos potencialmente envolvidos em interações bióticas exibidas pela marioila - *Phlomis purpurea* (Lamiaceae) em rizosferas infetadas por *P. cinnamomi*. Os compostos exsudados pelas raízes de *Phlomis purpurea* revelaram um efeito anti-*Phytophthora*.

(78)

Efeito supressivo de estrumes de origem animal no inóculo de *P. cinnamomi*

- Investigou-se o efeito de estrumes frescos e compostagem no desenvolvimento da podridão radicular causada por *P. cinnamomi*. O estrume proveniente de aviários reduziu significativamente a sobrevivência de *P. cinnamomi* e o desenvolvimento de sintomas em plantas jovens de tremoço branco (*Lupinus albus*), sobretudo com compostagem prolongada. Estrumes de bovinos, ovinos, caprinos e equinos, sujeitos ou não a compostagem, não suprimiram os sintomas de doença. Os estrumes de aviário compostados estimularam a atividade biológica e tiveram efeito supressivo em *P. cinnamomi*.

(79)

Referências bibliográficas

Brasier, C. M. (1999). The role *of Phytophthora* pathogens in forests and semi-natural communities in Europe and Africa. *In* Hansen, E.M. & Sutton, W. (eds.). *Phytophthora Diseases of Forest Trees*. Oregon, USA, 152 pp.

Coyle, D.R.; Klepzig, K.D.; Koch, F.H.; Morris, L.A.; Nowak, J.T.; Oak, S.W.; Otrosina, W.J.; Smith, W.D. & Gandhi, K.J.K. (2015). A review of southern pine decline in North America. *Forest Ecology and Management*, 349: 134-148.

Davison, E.M. (2018). Relative importance of site, weather and *Phytophthora cinnamomi* in the decline and death of *Eucalyptus marginata* – jarrah dieback investigations in the 1970s to 1990s. *Australasian Plant Pathology*, 47: 245–257.

FAO (2006). *Guidelines for Soil Description*. Fourth Edition. FAO, Rome, 109 pp.

ICNF (2019). *6º Inventário Florestal Nacional. Principais resultados. Relatório sumário. V1.0*. Junho 2019.

http://www2.icnf.pt/portal/florestas/ifn/resource/doc/ifn/IFN6-Principais resultados. Jun2019.pdf.

Journal Impact Factor (2017) – *Journal Citation Reports Science Edition* (Clarivate Analytics, 2019).

Maseko, B.; Burgess, T.I.; Coutinho, T.A. & Wingfield, M.J. (2007). Two new *Phytophthora* species from South African *Eucalyptus* plantations. *Mycological Research*, 111 (11): 1321-1338.

Pimentel, A.A.L. (1947). A *Phytophthora cinnamomi* Rands, um outro agente da tinta extremamente virulento da doença da tinta do castanheiro. *Agronomia Lusitana*, 9: 181-191.

Pulido, F. & Picardo, A. (2010). *Libro verde de la dehesa*. *Documento para el debate hacia una Estrategia Ibérica de Gestión*. Consejería de Medio Ambiente — Junta de Castilla y León, Sociedad Española de Ciencias Forestales, Sociedad Española para el Estudio de los Pastos, Asociación Española de Ecología Terrestre, Sociedad Española de Ornitología. 48 pp.

ANEXO I

Lista dos artigos científicos sobre *Phytophthora cinnamomi* em espécies de *Quercus* e do sobcoberto de montados/*dehesas*, por área temática - Quadros I a VIII, obtida por pesquisa na plataforma *WoS* em 11/05/2019. O número de citações refere-se a 2019 e o fator de impacto (IF) a 2017.

Quadro I – Artigos científicos sobre *Phytophthora cinnamomi* em *Quercus* spp. de montados/*dehesas*. Área Temática "**Fatores Bióticos – Agente Patogénico**"

Art. n.º	Ano	Autores	Título	Revista	URL	Nº cit.	IF
1	2018	Frisullo, S.; Lima, G.; San Lio, G.M.; Camele, I.; Melissano, L.; Puglisi, I.; Pane, A.; Agosteo, G.E.; Prudente, L. & Cacciola, S.O.	*Phytophthora cinnamomi* involved in the decline of holm oak (*Quercus ilex*) stands in Southern Italy	*Forest Science*, 64 (3): 290-298	https://doi.org/10.1093/forsci/fxx010	1	1,364
2	2018	Mora-Sala, B.; Berbegal, M. & Abad-Campos, P.	The use of qPCR reveals a high frequency of *Phytophthora quercina* in two spanish holm oak areas	*Forests*, 9 (11), 11697	https://doi.org/10.3390/f9110697	1	1,956
3	2015	Scanu, B.; Linaldeddu, B.T.; Deidda, A. & Jung, T.	Diversity of *Phytophthora* species from declining Mediterranean maquis vegetation, including two new species, *Phytophthora crassamura* and *P. ornamentata* sp. nov.	*PLoS ONE*, 10 (12): e0143234	https://doi.org/10.1371/journal.pone.0143234	14	2,766
4	2015	Serrano, M.S.; Rios, P.; Gonzalez, M. & Sanchez, M.E	Experimental minimum threshold for *Phytophthora cinnamomi* root disease expression on *Quercus suber*	*Phytopathologia Mediterranea*, 54 (3): 461-464	http://dx.doi.org/10.14601/Phytopathol_Mediterr-15128	6	1,442

Art. n.º	Ano	Autores	Título	Revista	URL	Nº cit.	IF
5	2014	Linaldeddu, B.T.; Scanu, B.; L. Maddau, L. & A. Franceschini	*Diplodia corticola* and *Phytophthora cinnamomi*: the main pathogens involved in holm oak decline on Caprera Island (Italy)	*Forest Pathology*, 44: 191–200	https://doi.org/10.1111/efp.12081	33	1,741
6	2013	Camilo-Alves, C.D.E.P.; da Clara, M.I.E. & Ribeiro, N.	Decline of Mediterranean oak trees and its association with *Phytophthora cinnamomi*: a review	*European Journal of Forest Research*, 132 (3): 411-432	https://doi.org/10.1007/s10342-013-0688-z	53	2,409
7	2013	Pérez-Sierra, A.; Lopez-Garcia, C.; León, M.; Garcia-Jiménez, J.; Abad-Campos, P. & T. Jung	Previously unrecorded low-temperature *Phytophthora* species associated with *Quercus* decline in a Mediterranean forest in eastern Spain	*Forest Pathology*, 43: 331–339	https://doi.org/10.1111/efp.12037	34	1,741
8	2012	Ruiz-Gomez, F.J.; Sanchez-Cuesta, R.; Navarro-Cerrillo, R.M. & Perez-de-Luque, A.	A method to quantify infection and colonization of holm oak (*Quercus ilex*) roots by *Phytophthora cinnamomi*	*Plant Methods*, 8: 39	https://doi.org/10.1186/1746-4811-8-39	4	4,269
9	2007	Romero, M.A.; Sanchez, J.E.; Jimenez, J.J.; Belbahri, L.; Trapero, A.; Lefort, F. & Sanchez, M.E.	New *Pythium taxa* causing root rot on Mediterranean *Quercus* species in South-west Spain and Portugal	*Journal of Phytopathology*, 155 (5): 289-295	https://doi.org/10.1111/j.1439-0434.2007.01230.x	40	0,823
10	2005	Sanchez, M.E.; Andicoberry, S. & Trapero, A.	Pathogenicity of three *Phytophthora* spp. causing late seedling rot of *Quercus ilex* ssp. *ballota*	*Forest Pathology*, 35 (2): 115-125	https://doi.org/10.1111/j.1439-0329.2004.00392.x	23	1,741

Art. n.º	Ano	Autores	Título	Revista	URL	Nº cit.	IF
11	2002	Sanchez, M.E.; Caetano, P.; Ferraz, J. & Trapero, A.	*Phytophthora* disease of *Quercus ilex* in south-western Spain	*Forest Pathology*, 32 (1): 5-18	https://doi.org/10.1046/j.1439-0329.2002.00261.x	90	1,741
12	2000	Luque, J.; Parlade, J. & Pera, J.	Pathogenicity of fungi isolated from *Quercus suber* in Catalonia (NE Spain)	*Forest Pathology*, 30 (5): 247-263	https://doi.org/10.1046/j.1439-0329.2000.00208.x	38	1,741
13	1999	Gallego, F.J.; de Algaba, A.P. & Fernandez-Escobar, R.	Etiology of oak decline in Spain	*European Journal of Forest Pathology*, 29 (1): 17-27	https://doi.org/10.1046/j.1439-0329.1999.00128.x	66	0,744
14	1999	Luque, J.; Cohen, M.; Save, R.; Biel, C. & Alvarez, I.F.	Effects of three fungal pathogens on water relations, chlorophyll fluorescence and growth of *Quercus suber* L.	*Annals of Forest Science*, 56 (1): 19-26	https://doi.org/10.1051/forest:19990103	33	0,936* (2002) 2,357 (2017)
15	1998	Robin, C.; Desprez-Loustau, M.L.; Capron, G. & Delatour, C.	First record of *Phytophthora cinnamomi* on cork and holm oaks in France and evidence of pathogenicity	*Annales des Sciences Forestières*, 55 (8): 869-883	https://doi.org/10.1051/forest:19980801	76	1,897** (2000)

* *Annals of Forest Science*, título da revista desde 1999. Entre 1964 e 1999 designada por *Annales des Sciences Forestières*.
IF relativo ao ano de 2002 é 0,936 e para o ano de 2017 é 2,357.

** *Annales des Sciences Forestières* , título da revista entre 1964 e 1999.
IF relativo ao ano de 2000 é 1,897.

Quadro II – Artigos científicos sobre *Phytophthora cinnamomi* em *Quercus* spp. de montados/*dehesas*. Área Temática "**Fatores Bióticos – Hospedeiro**"

Art. n.º	Ano	Autores	Título	Revista	URL	Nº cit.	IF
16	2019	Gallardo, A.; Morcuende, D.; Solla, A.; Moreno, G.; Pulido, F. & Quesada, A.	Regulation by biotic stress of tannins biosynthesis in *Quercus ilex*: Crosstalk between defoliation and *Phytophthora cinnamomi* infection	*Physiologia Plantarum*, 165 (2): 319-329	https://doi.org/10.1111/ppl.12848	1	2,58
17	2019	Maghnia, F.Z.; Abbas, Y.; Mahe, F.; Prin, Y.; El Ghachtouli, N.; Duponnois, R. & Sanguin, H.	The rhizosphere microbiome: A key component of sustainable cork oak forests in trouble	*Forest Ecology and Management*, 434: 29-39	https://doi.org/10.1016/j.foreco.2018.12.002	0	3,169
18	2018	Duque-Lazo, J.; Navarro-Cerrillo, R.M.; van Gils, H. & Groen, T.A.	Forecasting oak decline caused by *Phytophthora cinnamomi* in Andalusia: Identification of priority areas for intervention	*Forest Ecology and Management*, 417: 122-136	https://doi.org/10.1016/j.foreco.2018.02.045	2	3,169
19	2018	Moreira, A.C.; Tapias, R.; Fernandes, L. & Rodrigues, A.	Field susceptibility of cork oak trees with different provenances to *Phytophthora cinnamomi*	*Forest Pathology*, 48 (5), e12461	https://doi.org/10.1111/efp.12461	0	1,741
20	2017	Corcobado, T.; Miranda-Torres, J.J.; Martin-Garcia, J.; Jung, T. & Solla, A.	Early survival of *Quercus ilex* subspecies from different populations after infections and co-infections by multiple *Phytophthora* species	*Plant Pathology*, 66 (5): 792-804	https://doi.org/10.1111/ppa.12627	6	2,303

Art. n.º	Ano	Autores	Título	Revista	URL	Nº cit.	IF
21	2017	Leon, I.; Garcia, J.J.; Fernandez, M.; Vazquez-Pique, J. & Tapias, R.	Differences in root growth of *Quercus ilex* and *Quercus suber* seedlings infected with *Phytophthora cinnamomi*	*Silva Fennica*, 51 (4), 6991	https://doi.org/10.14214/sf.6991	2	1,683
22	2015	Corcobado, T.; Moreno, G.; Azul, A.M. & Solla, A.	Seasonal variations of ectomycorrhizal communities in declining *Quercus ilex* forests: interactions with topography, tree health status and *Phytophthora cinnamomi* infections	*Forestry*, 88 (2): 257-266	https://doi.org/10.1093/forestry/cpu056	12	2,638
23	2015	Ebadzad, G.; Medeira, C.; Maia, I.; Martins, J. & Cravador, A.	Induction of defence responses by cinnamomins against *Phytophthora cinnamomi* in *Quercus suber* and *Quercus ilex* subsp. *rotundifolia*	*European Journal of Plant Pathology*, 143 (4): 705-723	https://doi.org/10.1007/s10658-015-0721-9	4	1,466
24	2015	Redondo, M.A.; Perez-Sierra, A.; Abad-Campos, P.; Torres, L; Solla, A.; Reig-Arminana, J. & Garcia-Breijo, F.	Histology of *Quercus ilex* roots during infection by *Phytophthora cinnamomi*	*Trees-Structure and Function*, 29 (6): 1943-1957	https://doi.org/10.1007/s00468-015-1275-3	8	1,782
25	2015	Ruiz Gomez, F.J.; Navarro-Cerrillo, R.M.; Sanchez-Cuesta, R. & Perez-de-Luque, A.	Histopathology of infection and colonization of *Quercus ilex* fine roots by *Phytophthora cinnamomi*	*Plant Pathology*, 64 (3): 605-616	https://doi.org/10.1111/ppa.12310	6	2,303

Art. n.º	Ano	Autores	Título	Revista	URL	Nº cit.	IF
26	2014	Corcobado, T.; Vivas, M.; Moreno, G. & Solla, A.	Ectomycorrhizal symbiosis in declining and non-declining *Quercus ilex* trees infected with or free of *Phytophthora cinnamomi*	*Forest Ecology and Management*, 324: 72-80	http://dx.doi.org/10.1016/j.foreco.2014.03.040	15	3,169
27	2012	Medeira, C.; Quartin, V.; Maia, I.; Diniz, I.; Matos, M.C.; Semedo, J.N.; Scotti-Campos, P.; Ramalho, J.C.; Pais, I.P.; Ramos, P.; Melo, E.; Leitao, A.E. & Cravador, A.	Cryptogein and capsicein promote defence responses in *Quercus suber* against *Phytophthora cinnamomi* infection	*European Journal of Plant Pathology*, 134 (1): 145-159	https://doi.org/10.1007/s10658-012-9972-x	10	1,466
28	2012	Serrano, M.S.; De Vita, P.; Carbonero, M.D.; Fernandez, F.; Fernandez-Rebollo, P. & Sanchez, M.E.	Susceptibility to *Phytophthora cinnamomi* of the commonest morphotypes of holm oak in southern Spain	*Forest Pathology*, 42 (4): 345-347	https://doi.org/10.1111/j.1439-0329.2011.00758.x	7	1,741
29	2010	Horta, M.; Caetano, P.; Medeira, C.; Maia, I. & Cravador, A.	Involvement of the β-cinnamomin elicitin in infection and colonisation of cork oak roots by *Phytophthora cinnamomi*	*European Journal of Plant Pathology*, 127 (3): 427-436	https://doi.org/10.1007/s10658-010-9609-x	14	1,466
30	2009	Moralejo, E.; Garcia-Munoz, J.A. & Descals, E.	Susceptibility of Iberian trees to *Phytophthora ramorum* and *P. cinnamomi*	*Plant Pathology*, 58 (2): 271-283	https://doi.org/10.1111/j.1365-3059.2008.01956.x	29	2,303
31	2008	Maia, I.; Medeira, C.; Melo, E. & Cravador, A.	*Quercus suber* infected by *Phytophthora cinnamomi* effects at cellular level of cinnamomin on roots, stem and leaves	*Microscopy and Microanalysis*, 14: 146-147, Supplement: 3	https://doi.org/10.1017/S1431927608089708	3	2,124

Art. n.º	Ano	Autores	Título	Revista	URL	Nº cit.	IF
32	2005	Rodriguez-Molina, M.C.; Blanco-Santos, A.; Palo-Nunez, E.J.; Torres-Vila, L.M.; Torres-Alvarez, E. & Suarez-de-la-Camara, M.A.	Seasonal and spatial mortality patterns of holm oak seedlings in a reforested soil infected with *Phytophthora cinnamomi*	*Forest Pathology*, 35 (6): 411-422	https://doi.org/10.1111/j.1439-0329.2005.00423.x	12	1,741
33	2002	Rodriguez-Molina, M.C.; Torres-Vila, L.M.; Blanco-Santos, A.; Nunez, E.J.P. & Torres-Alvarez, E.	Viability of holm and cork oak seedlings from acorns sown in soils naturally infected with *Phytophthora cinnamomi*	*Forest Pathology*, 32 (6): 365-372	https://doi.org/10.1046/j.1439-0329.2002.00297.x	15	1,741
34	2001	Maurel, M.; Robin, C.; Capron, G. & Desprez-Loustau, M.L.	Effects of root damage associated with *Phytophthora cinnamomi* on water relations, biomass accumulation, mineral nutrition and vulnerability to water deficit of five oak and chestnut species	*Forest Pathology*, 31 (6): 353-369	https://doi.org/10.1046/j.1439-0329.2001.00258.x	36	1,741
35	2001	Robin, C.; Capron, G. & Desprez-Loustau, M.L.	Root infection by *Phytophthora cinnamomi* in seedlings of three oak species	*Plant Pathology*, 50 (6): 708-716	https://doi.org/10.1046/j.1365-3059.2001.00643.x	51	2,303
36	1992	Brasier, C.M.; Robredo, F. & Ferraz, J.F.P.	Evidence for *Phytophthora cinnamomi* involvement in Iberian oak decline	*Plant Pathology*, 42 (1): 140-145	https://doi.org/10.1111/j.1365-3059.1993.tb01482.x	166	2,303

Quadro III – Artigos científicos sobre *Phytophthora cinnamomi* em *Quercus* spp. de montados/*dehesas*. Área Temática "**Fatores Abióticos**"

Art. n.º	Ano	Autores	Título	Revista	URL	Nº cit.	IF
37	2018	Gomez, F.J.R.; Perez-de-Luque, A.; Sanchez-Cuesta, R.; Quero, J.L. & Cerrillo, R.M.N.	Differences in the response to acute drought and *Phytophthora cinnamomi* Rands infection in *Quercus ilex* L. seedlings	*Forests*, 9, 634, 16pp.	http://hdl.handle.net/10396/17441	1	1,956
38	2018	Hernandez-Lambrano, R.E.; Gonzalez-Moreno, P. & Sanchez-Agudo, J.A.	Environmental factors associated with the spatial distribution of invasive plant pathogens in the Iberian Peninsula: The case of *Phytophthora cinnamomi* Rands	*Forest Ecology and Management*, 419: 101-109	https://doi.org/10.1016/j.foreco.2018.03.026	2	3,169
39	2015	Martin-Garcia, J.; Solla, A.; Corcobado, T.; Siasou, E. & Woodward, S.	Influence of temperature on germination of *Quercus ilex* in *Phytophthora cinnamomi*, *P. gonapodyides*, *P. quercina* and *P. psychrophila* infested soils	*Forest Pathology*, 45 (3): 215-223	https://doi.org/10.1111/efp.12159	11	1,741
40	2014	Corcobado, T.; Cubera, E.; Juarez, E.; Moreno, G. & Solla, A.	Drought events determine performance of *Quercus ilex* seedlings and increase their susceptibility to *Phytophthora cinnamomi*	*Agricultural and Forest Meteorology*, 192: 1-8	http://dx.doi.org/10.1016/j.agrformet.2014.02.007	31	4,039
41	2013	Corcobado, T.; Cubera, E.; Moreno, G. & Solla, A.	*Quercus ilex* forests are influenced by annual variations in water table, soil water deficit and fine root loss caused by *Phytophthora cinnamomi*	*Agricultural and Forest Meteorology*, 169: 92-99	https://doi.org/10.1016/j.agrformet.2012.09.017	37	4,039

Art. n.º	Ano	Autores	Título	Revista	URL	Nº cit.	IF
42	2005	Moreira, A.C. & Martins, J.M.S.	Influence of site factors on the impact of *Phytophthora cinnamomi* in cork oak stands in Portugal	*Forest Pathology*, 35 (3): 145-162	https://doi.org/10.1111/j.1439-0329.2005.00397.x	62	1,741
43	2004	Turco, E.; Close, T.J.; Fenton, R.D. & Ragazzi, A.	Synthesis of dehydrin-like proteins in *Quercus ilex* L. and *Quercus cerris* L. seedlings subjected to water stress and infection with *Phytophthora cinnamomi*	*Physiological and Molecular Plant Pathology*, 65 (3): 137-144	https://doi.org/10.1016/j.pmpp.2004.11.010	14	1,395
44	2002	Luque, J.; Parlade, J. & Pera, J.	Seasonal changes in susceptibility of *Quercus suber* to *Botryosphaeria stevensii* and *Phytophthora cinnamomi*	*Plant Pathology*, 51 (3): 338-345	https://doi.org/10.1046/j.1365-3059.2002.00713.x	18	2,303
45	1996	Brasier, C.M.	*Phytophthora cinnamomi* and oak decline in southern Europe. Environmental constraints including climate change	*Annales des Sciences Forestières*, 53 (2-3): 347-358	https://doi.org/10.1051/forest:19960217	202	1,897* (2000)

* *Annales des Sciences Forestières* , título da revista entre 1964 e 1999.
IF relativo ao ano de 2000 é 1,897.

Quadro IV – Artigos científicos sobre *Phytophthora cinnamomi* em *Quercus* spp. de montados/*dehesas*. Área Temática "**Biologia Molecular/Bioquímica/Fisiologia**"

Pub. n.º	Ano	Autores	Título	Revista	URL	Nº cit.	IF
46	2019	Gómez, F.J.R.; Navarro-Cerrillo, R.M.: Pérez-de-Luque, A.; Oβwald, W.: Vannini, A.& Morales-Rodríguez, C.	Assessment of functional and structural changes of soil fungal and oomycete communities in holm oak declined *dehesas* through metabarcoding analysis	*Scientific Reports*, 9:5315, 16 pp.	https://doi.org/10.1038/s41598-019-41804-y	0	4,122
47	2018	Cernadas,M.J.; Martínez, M.T. Corredoira, E. & San-José, M.C.	Conservation of holm oak (*Quercus ilex*) by *in vitro* culture	*Mediterranean Botany,* 39 (2): 97-104	http://dx.doi.org/10.5209/MBOT.60779	1	*
48	2017	Balde, A.; Neves, D.; Garcia-Breijo, F.J.; Pais, M.S. & Cravador, A.	De novo assembly of *Phlomis purpurea* after challenging with *Phytophthora cinnamomi*	*BMC Genomics*, 18: 700	https://doi.org/10.1186/s12864-017-4042-6	0	3,73
49	2017	Catala, S.; Berbegal, M.; Perez-Sierra, A. & Abad-Campos, P.	Metabarcoding and development of new real-time specific assays reveal *Phytophthora* species diversity in holm oak forests in eastern Spain	*Plant Pathology*, 66 (1): 115-123	https://doi.org/10.1111/ppa.12541	8	2,303
50	2016	Cimmino, A.; Maddau, L.; Masi, M.; Evidente, M.; Linaldeddu, B.T. & Evidente, A.	Further secondary metabolites produced by *Diplodia corticola*, a fungal pathogen involved in cork oak decline	*Tetrahedron*, 72 (43): 6788-6793	https://doi.org/10.1016/j.tet.2016.09.008	6	2,377

*Revista ainda sem IF (anteriormente designada *Lazaroa*).

Pub. n.º	Ano	Autores	Título	Revista	URL	Nº cit.	IF
51	2016	Hardoim, P.R.; Guerra, R.; da Costa, A.M.; Serrano, M.S.; Sanchez, M.E. & Coelho, A.C.	Temporal metabolic profiling of the *Quercus suber–Phytophthora cinnamomi* system by middle-infrared spectroscopy	*Forest Pathology*, 46 (2): 122-133	https://doi.org/10.1111/efp.12229	3	1,741
52	2014	Ebadzad, G. & Cravador, A.	Quantitative RT-PCR analysis of differentially expressed genes in *Quercus suber* in response to *Phytophthora cinnamomi* infection	*SpringerPlus*, 3, 613, 13pp	https://doi.org/10.1186/2193-1801-3-613	9	1,13
53	2013	Machado, A.; Pereira, H. & Teixeira, R.T.	Anatomy and development of the endodermis and phellem of *Quercus suber* L. roots	*Microscopy and Microanalysis*, 19 (3): 525-534	https://doi.org/10.1017/S1431927613000287	2	2,124
54	2013	Sghaier-Hammami, B.; Valero-Galvan, J.; Romero-Rodriguez, M.C.; Navarro-Cerrillo, R.M.; Abdelly, C. & Jorrin-Novo, J.	Physiological and proteomics analyses of holm oak (*Quercus ilex* subsp. *ballota* [Desf.] Samp.) responses to *Phytophthora cinnamomi*	*Plant Physiology and Biochemistry*, 71: 191-202	https://doi.org/10.1016/j.plaphy.2013.06.030	22	2,718
55	2008	Horta, M.; Sousa, N.; Coelho, A.C.; Neves, D. & Cravador, A.	*In vitro* and *in vivo* quantification of elicitin expression in *Phytophthora cinnamomi*	*Physiological and Molecular Plant Pathology*, 73 (1-3): 48-57	https://doi.org/10.1016/j.pmpp.2009.02.003	11	1,395
56	2006	Coelho, A.C.; Horta, M.; Neves, D. & Cravador, A.	Involvement of a cinnamyl alcohol dehydrogenase of *Quercus suber* in the defence response to infection by *Phytophthora cinnamomi*	*Physiological and Molecular Plant Pathology*, 69 (1-3): 62-72	https://doi.org/10.1016/j.pmpp.2007.01.001	9	1,395

Quadro V – Artigos científicos sobre *Phytophthora cinnamomi* em *Quercus* spp. de montados/*dehesas*. Área Temática "**Prevenção e Controlo**"

Art. n.º	**Ano**	**Autores**	**Título**	**Revista**	**URL**	**Nº cit.**	**IF**
57	2017	Gonzalez, M.; Caetano, P. & Sanchez, M.E.	Testing systemic fungicides for control of *Phytophthora* oak root disease	*Forest Pathology*, 47 (4): e12343	https://doi.org/10.1111/efp.12343	1	1,741
58	2016	Rios, P.; Obregon, S.; Gonzalez, M.; de Haro, A. & Sanchez, M.E.	Screening brassicaceous plants as biofumigants for management of *Phytophthora cinnamomi* oak disease	*Forest Pathology*, 46 (6): 652-659	https://doi.org/10.1111/efp.12287	5	1,741
59	2016	Rios, P.; Obregon, S.; de Haro, A.; Fernandez-Rebollo, P.; Serrano, M.S. & Sanchez, M.E.	Effect of *Brassica* biofumigant amendments on different stages of the life cycle of *Phytophthora cinnamomi*	*Journal of Phytopathology*, 164 (9): 582-594	https://doi.org/10.1111/jph.12482	4	0,823
60	2014	Neves, D.; Caetano, P.; Oliveira, J.; Maia, C.; Horta, M.; Sousa, N.; Salgado, M.; Dionisio, L.; Magan, N. & Cravador, A.	Anti-*Phytophthora cinnamomi* activity of *Phlomis purpurea* plant and root extracts	*European Journal of Plant Pathology*, 138 (4): 835-846	https://doi.org/10.1007/s10658-013-0357-6	3	1,466
61	2013	Serrano, M.S.; Fernandez-Rebollo, P.; de Vita, P. & Sanchez, M.E.	Calcium mineral nutrition increases the tolerance of *Quercus ilex* to *Phytophthora* root disease affecting oak rangeland ecosystems in Spain	*Agroforestry Systems*, 87 (1): 173-179	https://doi.org/10.1007/s10457-012-9533-5	5	1,201

Art. n.º	Ano	Autores	Título	Revista	URL	Nº cit.	IF
62	2012	Serrano, M.S.; de Vita, P.; Fernandez-Rebollo, P. & Hernandez, M.E.	Calcium fertilizers induce soil suppressiveness to *Phytophthora cinnamomi* root rot of *Quercus ilex*	*European Journal of Plant Pathology*, 132 (2): 271-279	https://doi.org/10.1007/s10658-011-9871-6	17	1,466
63	2009	Solla, A.; Garcia, L.; Perez, A.; Cordero, A.; Cubera, E. & Moreno, G.	Evaluating potassium phosphonate injections for the control of *Quercus ilex* decline in SW Spain: implications of low soil contamination by *Phytophthora cinnamomi* and low soil water content on the effectiveness of treatments	*Phytoparasitica*, 37 (4): 303-316	https://doi.org/10.1007/s12600-009-0042-7	10	1,007
64	2009	Vicente, M.; Sanchez, M.; Fernandez, P. & Trapero, A.	Evaluation of biofumigant plants and organic amendments for suppressiveness of root rot of *Quercus* spp. caused by *Phytophthora cinnamomi*	*Phytopathology*, 99 (6): S134-S135	https://apsjournals.apsnet.org/doi/pdf/10.1094/PHYTO.2009.99.6.S1	0	3,036
65	1999	Fernandez-Escobar, R.; Gallego, F.J.; Benlloch, M.; Membrillo, J.; Infante, J. & de Algaba, A.P.	Treatment of oak decline using pressurized injection capsules of antifungal materials	*European Journal of Forest Pathology*, 29 (1): 29-38	https://doi.org/10.1046/j.1439-0329.1999.00128.x	20	0,744

Quadro VI – Artigos científicos sobre *Phytophthora cinnamomi* em *Quercus* spp. de montados/*dehesas*. Área Temática "**Ecologia**"

Art. n.º	Ano	Autores	Título	Revista	URL	Nº cit.	IF
66	2019	Matías, L.; Abdelaziz, M.; Godoy, O. & Gómez-Aparicio, L.	Disentangling the climatic and biotic factors driving changes in the dynamics of *Quercus suber* populations across the species' latitudinal range	*Diversity and Distributions*, 25:524–535	https://onlinelibrary.wiley.com/doi/abs/10.1111/ddi.12873	1	4,614
67	2018	Sena, K.; Crocker, E.; Vincelli, P. & Barton, C. .	*Phytophthora cinnamomi* as a driver of forest change: Implications for conservation and management	*Forest Ecology and Management*, 409: 799-807	https://doi.org/10.1016/j.foreco.2017.12.022	8	3,169
68	2017	Avila, J.M.; Linares, J.C.; Garcia-Nogales, A.; Sanchez, M.E. & Gomez-Aparicio, L.	Across-scale patterning of plant–soil–pathogen interactions in *Quercus suber* decline	*European Journal of Forest Research*, 136 (4): 677-688	http://dx.doi.org/10.1007/s10342-017-1064-1	2	2,409
69	2017	Gomez-Aparicio, L.; Dominguez-Begines, J.; Kardol, P.; Avila, J.M.; Ibanez, B. & Garcia, L.V.	Plant-soil feedbacks in declining forests: implications for species coexistence	*Ecology*, 98 (7): 1908-1921	https://doi.org/10.1002/ecy.1864	5	4,617

Quadro VII – Artigos científicos sobre *Phytophthora cinnamomi* em *Quercus* spp. de montados/*dehesas*. Área Temática "**Solo**"

Art. n.º	Ano	Autores	Título	Revista	URL	Nº cit.	IF
70	2019	Avila, J.M.; Gallardo, A. & Gomez-Aparicio, L.	Pathogen-induced tree mortality interacts with predicted climate change to alter soil respiration and nutrient availability in Mediterranean systems	*Biochemistry*, 142 (1): 53-71	http://dx.doi.org/10.1007/s10533-018-0521-3	0	3,265
71	2018	Jimenez-Chacon, A.; Homet, P.; Matias, L.; Gomez-Aparicio, L. & Godoy, O.	Fine scale determinants of soil litter fauna on a Mediterranean mixed oak forest invaded by the exotic soil-borne pathogen *Phytophthora cinnamomi*	*Forests*, 9 (4), 218, 16pp.	https://doi.org/10.3390/f9040218	0	1,956
72	2016	Avila, J.M.; Gallardo, A.; Ibanez, B. & Gomez-Aparicio, L.	*Quercus suber* dieback alters soil respiration and nutrient availability in Mediterranean forests	*Journal of Ecology*, 104 (5): 1441-1452	https://doi.org/10.1111/1365-2745.12618	7	5,172
73	2013	Corcobado, T.; Solla, A.; Madeira, M.A. & Moreno, G.	Combined effects of soil properties and *Phytophthora cinnamomi* infections on *Quercus ilex* decline	*Plant and Soil*, 373 (1-2): 403-413	https://doi.org/10.1007/s11104-013-1804-z	15	3,306
74	2012	Gomez-Aparicio, L.; Ibanez, B.; Serrano, M.S.; de Vita, P.; Avila, J.M.; Perez-Ramos, I.M.; Garcia, L.V.; Sanchez, M.E. & Maranon, T.	Spatial patterns of soil pathogens in declining Mediterranean forests: implications for tree species regeneration	*New Phytologist*, 194 (4): 1014-1024	https://doi.org/10.1111/j.1469-8137.2012.04108.x	40	7,433

Quadro VIII – Artigos científicos sobre *Phytophthora cinnamomi* em espécies do sobcoberto de montados/*dehesas:* Área Temática "**Sobcoberto**"

Art. n.º	Ano	Autores	Título	Revista	URL	Nº cit.	IF
75	2012	Serrano, M.S.; Fernandez-Rebollo, P.; De Vita, P. & Sanchez, M.E.	Susceptibility of common herbaceous crops to *Phytophthora cinnamomi* and its influence on *Quercus* root rot in rangelands	*European Journal of Plant Pathology*, 134 (2): 409-414	https://doi.org/10.1007/s10658-012-9999-z	5	1,466
76	2011	Serrano, M.S.; Fernandez-Rebollo, P.; De Vita, P.; Carbonero, M.D. & Sanchez, M.E.	The role of yellow lupin (*Lupinus luteus*) in the decline affecting oak agroforestry ecosystems	*Forest Pathology*, 41 (5): 382-386	https://doi.org/10.1111/j.1439-0329.2010.00694.x	3	1,741
77	2010	Serrano, M.S.; Fernandez-Rebollo, P.; De Vita, P.; Carbonero, M.D.; Trapero, A. & Sanchez, M.E.	*Lupinus luteus*, a new host of *Phytophthora cinnamomi* in Spanish oak-rangeland ecosystems	*European Journal of Plant Pathology*, 128 (2): 149-152	https://doi.org/10.1007/s10658-010-9652-7	12	1,466
78*	2016	Mateus, M.C.; Neves, D.; Dacunha, B.; Laczko, E.; Maia, C.; Teixeira, R. & Cravador, A.	Structure, anti-*Phytophthora* and anti-tumor activities of a nortriterpenoid from the rhizome of *Phlomis purpurea* (Lamiaceae)	*Phytochemistry*, 131: 158-164	http://dx.doi.org/10.1016/j.phytochem.2016.09.004	2	3.186
79*	2000	Aryantha, I.P.; Cross, R. & Guest, D.I.	Suppression of *Phytophthora cinnamomi* in potting mixes amended with uncomposted and composted animal manures	*Phytopathology,* 90 (7): 775-782	https://doi.org/10.1094/PHYTO.2000.90.7.775	57	3,036

* Artigos devolvidos na 2ª fase da pesquisa.

ANEXO II

Título e resumo dos artigos científicos sobre *Phytophthora cinnamomi* em espécies de *Quercus* e do sobcoberto de montados e *dehesas* apresentados por área temática dominante (pesquisa em 11/05/2019 na plataforma *WoS*).

Artigo n.º **Título**/Resumo

1 ***Phytophthora cinnamomi* Involved in the decline of holm oak (*Quercus ilex*) stands in southern Italy**

During a survey of forest stands of holm oak (*Quercus ilex*) in the Salento peninsula, Apulia region (southern Italy), the oomycete *Phytophthora cinnamomi* was found to be consistently associated with tree decline and mortality in 7 municipalities of the province of Lecce. The pathogen was recovered directly from roots using a selective medium and from rhizosphere soil samples with leaf baits and subsequent isolation on the same selective medium used for direct isolation from roots. It was identified on the basis of morphological characters and by sequencing of the internal transcribed spacer (ITS) regions of the rDNA after amplification with conventional PCR. All *P. cinnamomi* isolates were A2 mating type and proved to be highly aggressive on seedlings of evergreen Mediterranean oak species, including holm oak, cork oak (*Q. suber*) and kermes oak (*Q. coccifera*). *P. cinnamomi* is a well know pathogen of several forest trees worldwide and on the basis of its widespread and consistent occurrence in forest stands of the Lecce province it was assumed to be the primary causal agent of holm oak decline in this area. Options for the management of this phytosanitary environmental emergence are discussed.

2 **The use of qPCR reveals a high frequency of *Phytophthora quercina* in two Spanish holm oak areas**

The struggling Spanish holm oak woodland situation associated with *Phytophthora* root rot has been studied for a long time. *Phytophthora cinnamomi* is considered the main, but not the only species responsible for the decline scenario. This study verifies the presence and/or detection of *Phytophthora* species in two holm oak areas of Spain (southwestern *dehesas* and northeastern woodland) using different isolation and detection approaches. Direct isolation and baiting methods in declining and non-declining holm oak trees revealed *Phytophthora cambivora*, *Phytophthora cinnamomi*, *Phytophthora gonapodyides*, *Phytophthora megasperma*, and *Phytophthora pseudocryptogea* in the dehesas, while in northeastern woodland, no *Phytophthora* spp. were recovered. *Phytophthora quercina* and *P. cinnamomi* TaqMan real-time PCR probes showed that both *P. cinnamomi* and *P. quercina* are involved in the holm oak decline in Spain, but *P. quercina* was detected in a higher frequency than *P. cinnamomi* in both studied areas. Thus, this study demonstrates that molecular approaches complement direct isolation techniques in natural and seminatural ecosystem surveys to determine the presence and distribution of *Phytophthora* spp. This is the first report of *P. pseudocryptogea* in Europe and its role in the holm oak decline should be further studied.

3 Diversity of *Phytophthora species* from declining Mediterranean maquis vegetation, including two new species, *Phytophthora crassamura* and *P. ornamentata* sp. nov.

The Mediterranean basin is recognized as a global biodiversity hotspot accounting for more than 25,000 plant species that represent almost 10% of the world's vascular flora. In particular, the maquis vegetation on Mediterranean islands and archipelagos constitutes an important resource of the Mediterranean plant diversity due to its high rate of endemism. Since 2009, a severe and widespread dieback and mortality of *Quercus ilex* trees and several other plant species of the Mediterranean maquis has been observed in the National Park of La Maddalena archipelago (northeast Sardinia, Italy). Infected plants showed severe decline symptoms and a significant reduction of natural regeneration. First studies revealed the involvement of the highly invasive wide-host range pathogen *Phytophthora cinnamomi* and several fungal pathogens. Subsequent detailed research led to a better understanding of these epidemics showing that multiple *Phytophthora* spp. were involved, some of them unknown to science. In total, nine *Phytophthora* species were isolated from rhizosphere soil samples collected from around symptomatic trees and shrubs including *Asparagus albus*, *Cistus* sp., *Juniperus phoenicea*, *J. oxycedrus*, *Pistacia lentiscus* and *Rhamnus alaternus*. Based on morphological characters, growth-temperature relations and sequence analysis of the ITS and cox1 gene regions, the isolates were identified as *Phytophthora asparagi*, *P. bilorbang*, *P. cinnamomi*, *P. cryptogea*, *P. gonapodyides*, *P. melonis*, *P. syringae* and two new Clade 6 *taxa* which are here described *as P. crassamura* sp. nov. and *P. ornamentata* sp. nov. Pathogenicity tests supported their possible involvement in the severe decline that is currently threatening the Mediterranean maquis vegetation in the La Maddalena archipelago.

4 Experimental minimum threshold for *Phytophthora cinnamomi* root disease expression on *Quercus suber*

Quercus suber seedlings were potted in soils infested with increasing concentrations of *Phytophthora cinnamomi* chlamydospores and submitted to weekly flooding for 3 months to favour root infections. Increasing quantities of chlamydospores led to an exponential increase in their ability to germinate. Root symptoms (necrosis and/or absence of feeder roots) were significantly more severe than those recorded in uninfested soil only for plants potted in soils infested with 61 cfu g^{-1} or more. Although generated using potting mix, this minimum threshold represents a tool for checking the potential infectivity of infested soils or to assess the effectiveness of some control methods to reduce soil inoculum. However, a low level of root infection was recorded even at 3 cfu g^{-1}. Therefore, long-term disease risk may be present whenever the pathogen is detectable in oak forest soils.

5 *Diplodia corticola* and *Phytophthora cinnamomi*: the main pathogens involved in holm oak decline on Caprera Island (Italy)

Since 2008, severe and widespread tree decline and mortality has been observed at the main growing *Quercus ilex* L. (holm oak) forest on Caprera Island, Italy. To clarify the symptomatology and aetiology of this phenomenon, field surveys and isolations from symptomatic trees were carried out in summer 2010. Affected trees exhibited crown thinning, branch dieback, sunken cankers, epicormic shoots, exudates on branches and trunk, root losses and sudden death symptoms. Four fungal species belonging to Botryosphaeriaceae family, namely *Botryosphaeria dothidea*, *Diplodia corticola*, *D. seriata* and *Neofusicoccum parvum*, were isolated from cankers on trunk and branches, whereas three species of *Phytophthora*, namely *P. cinnamomi*, *P. cryptogea* and *P. gonapodyides*, were isolated from fine roots and rhizosphere soil samples. Isolates were identified using both morphological analysis and DNA-based techniques. Pathogenicity trials on holm oak seedlings showed that all the isolated species are pathogenic. *D. corticola* proved to be the most aggressive species. Our results provide the first evidence for a combined involvement of *D. corticola* and *P. cinnamomi* in the aetiology of holm oak decline in Italy and suggest that these pathogens are not only important contributing factors in the onset of long-term tree decline, but also may cause the rapid devastation of extensive oak ecosystems.

6 **Decline of Mediterranean oak trees and its association with *Phytophthora cinnamomi*: a review**

Mortality events in cork and holm oaks have occurred in the Mediterranean basin since the beginning of the XX century, but severity of decline increased during the 1980s. By that time, the exotic soil borne pathogen *Phytophthora cinnamomi* was often recovered from declining stands and since then it has been considered the main factor associated with decline. This work analyses data concerning *P. cinnamomi* surveys in cork and holm oaks trees, pathogenicity tests carried out in controlled experiments, studies about the influence of site characteristics in tree decline and approaches to control the disease. Results of field surveys showed that the pathogen is widespread and pathogenicity tests suggested that host susceptibility to the pathogen is moderate when seedlings are in appropriate watering conditions, particularly cork oaks. Occurrence of decline is also associated with soil characteristics that interfere with root expansion and water retention. We assessed the relative importance of each factor involved in decline and revised the role of *P. cinnamomi* in cork and holm oak decline.

7 **Previously unrecorded low-temperature *Phytophthora* species associated with *Quercus decline* in a Mediterranean forest in eastern Spain**

Oak decline has been a serious problem in Europe since the beginning of the twentieth century. In south-west Spain, *Quercus ilex* and *Q. suber* are the main affected species, and their decline has been associated with *Phytophthora cinnamomi*. During the last 10 years, a severe decline of *Q. ilex* and *Q. faginea* accompanied by a significant decrease in the production of acorns affecting natural regeneration was observed in the eastern part of the Iberian Peninsula. Therefore, the aim of this study was to investigate the possible involvement of *Phytophthora* spp. in the decline. A forest in the Natural Park 'Carrascar de la Font Roja' in Comunidad Valenciana (eastern Spain), which is dominated by *Q. ilex* and *Q*. *faginea*, was surveyed during 2010–2011. Symptomatic trees showed thinning and dieback of the crown, withering of leaves and death. An extensive loss of both lateral small woody roots and fine roots and callusing or open cankers on suberized roots were observed. Soil samples containing fine roots were baited using both *Q. robur* leaves and apple fruits. Six *Phytophthora* species were isolated: *P. cryptogea*, *P. gonapodyides*, *P. megasperma*, *P. quercina*, *P. psychrophila* and *P. syringae*. These are the first records of *P. quercina* and *P. psychrophila* on *Q. faginea*, of *P. quercina* in Spain and of *P. psychrophila* in mainland Spain. A soil infestation trial was conducted for 6 months under controlled conditions with 1-year-old seedlings of *Q. ilex* and *Q. faginea*. *Phytophthora cinnamomi* was included in the pathogenicity test for comparison. The results showed that *Q. ilex* seedlings were generally more susceptible to infection than *Q. faginea* with *P. cinnamomi* being the most aggressive pathogen to both oak species. The two most commonly isolated *Phytophthora* species, *P. quercina* and *P. psychrophila*, also proved their pathogenicity towards both *Q. ilex* and *Q. faginea*.

8 A method to quantify infection and colonization of holm oak (*Quercus ilex*) roots by *Phytophthora cinnamomi*

Phytophthora cinnamomi Rands. is an important root rot pathogen widely distributed in the north hemisphere, with a large host range. Among others diseases, it is known to be a principal factor in the decline of holm oak and cork oak, the most important tree species in the "dehesa" ecosystem of south-western Spain. Previously, the focus of studies on *P. cinnamomi* and holm oak have been on molecular tools for identification, functional responses of the host, together with other physiological and morphological host variables. However, a microscopic index to describe the degree of infection and colonization in the plant tissues has not yet been developed. A colonization or infection index would be a useful tool for studies that examine diferences between individuals subjected to different treatments or to individuals belonging to different breeding accessions, together with their specific responses to the pathogen. This work presents a methodology based on the capture and digital treatment of microscopic images, using simple and accessible software, together with a range of variables that quantify the infection and colonization process.

9 New *Pythium taxa* causing root rot on Mediterranean *Quercus* species in South-west Spain and Portugal

Pythium spiculum, a recently described new taxon, has been frequently isolated from declining *Quercus rotundifolia* and *Q. suber* roots and rhizosphere since 2003 in southern Iberia. In soils of declining *Quercus* forests this species was found as frequently as *Phytophthora cinnamomi* which, until now, was the only oomycete described as a *Quercus* root rot pathogen in the region. Inoculation tests conducted on young *Q.*

rotundifolia plants showed that *Py. spiculum* is an aggressive root pathogen, although producing severities of symptoms significantly lower than those of *P. cinnamomi*. This new pathogen could play a role as decline factor in southern Iberia. Another new species, *Py. sterilum*, was also found to be pathogenic to *Quercus* roots but there are presently only few records of this organism isolated from rhizosphere of declining oaks in central Spain. More than an active decline factor, this species should be considered as a potential risk for *Quercus* forests.

10 Pathogenicity of three *Phytophthora* spp. causing late seedling rot of *Quercus ilex* ssp. *ballota*

Within a research project on quality of plants for forestation of agricultural lands, we studied the aetiology of a late seedling rot affecting holm oak (*Quercus ilex* ssp. *ballota*) in two forest nurseries in southern Spain. Major disease symptoms were foliage wilting and necrosis of feeder roots. *Phytophthora cinnamomi*, *Phytophthora cryptogea* and *Phytophthora drechsleri* were isolated from necrotic roots of holm oaks. Selected isolates of the three *Phytophthora* species were pathogenic to *Quercus ilex* ssp. *ballota* and *Quercus suber* seedlings in artificial inoculations. Soil flooding conditions were essential for infection and root rot development. There was no host specificity among the species, the isolates of *P. cinnamomi* being the most virulent in all inoculated plants. In these inoculations, *Q. ilex* ssp. *ballota* plants were more susceptible than those of *Q. suber*. This work is the first report of *P. cinnamomi*, *P. drechsleri* and *P. cryptogea* affecting *Q. ilex* ssp. *ballota* in forest nurseries.

11 *Phytophthora* disease of *Quercus ilex* in south-western Spain

Oak decline that was affecting three holm oak sites in the province of Huelva (south-western Spain) was studied during 1998-1999. The syndromes of dieback and sudden death have been observed and, in both cases, foliar symptoms were associated with root rot. Characterization of the fungal isolates from necrotic roots led us to identify *Phytophthora cinnamomi* A2 as consistently associated with the disease. The optimum growth temperatures of these isolates were very high (30°C). Inoculation tests under controlled conditions demonstrated the pathogenicity of the isolates on holm and cork oak seedlings. None of the other biotic factors of Mediterranean oak decline that have been previously described were found in the present study and so, in this case, the forest decline model does not seem to be necessary in order to explain the disease observed. The defoliation and mortality of the oaks was primarily caused by *P. cinnamomi*, although sonic abiotic factors such as alternating periods of drought and wet weather in the region may play an important role.

12 Pathogenicity of fungi isolated from *Quercus suber* in Catalonia (NE Spain)

Thirty-four fungal species isolated from cork oak (*Quercus suber*) in Catalonia (NE Spain) during 1992-95 were tested for pathogenicity either in stem, leaf or root inoculations. Eleven species were found to be pathogenic on stem: *Biscogniauxia mediterranea*, *Botryosphaeria stevensii*, *Diatrype* cf. *stigma*, *Endothia gyrosa*, *Fusarium solani*, *Graphium* sp., *Ophiostoma quercus*, *Phomopsis* sp., *Phytophthora cinnamomi*, *Sporendocladia bactrospora* and an unidentified Coelomycete. Three fungi showed pathogenic effects on leaves: *Dendrophoma myriadea*, *Lembosia quercina* and *Phomopsis quercella*. No clear pathogenic effects were detected in the root inoculation experiment. Trunk pathogens were differentiated into two groups according to the effects induced in the inoculated plants; *B. stevensii*, *Phomopsis* sp. and *P. cinnamomi* caused the death of the inoculated plants and induced the formation of large cankers and vascular necroses. The other pathogenic species also produced severe cankers and vascular lesions, but no significant mortality was detected. Water stress increased the lesions caused by *B. mediterranea* and *Phomopsis* sp., but limited those of *P. cinnamomi* and the rest of the inoculated fungi. However, water stress did not significantly affect the damage caused by *B. stevensii*, which was the most virulent of the species tested. Leaf pathogens only showed their effects if the leaf cuticle was previously damaged. *Lembosia quercina* caused small dark lesions whereas *D. myriadea* and *P. quercella* produced large necrotic areas in wellwatered plants. The lesions caused by the last two fungi were reduced by water stress.

13 Etiology of oak decline in Spain

In different areas of Extremadura, Western Spain, soil samples were taken at the bottom of holm oak (*Quercus ilex*) trees that were showing decline symptoms. Half of each sample was sterilized, and acorns were sown in both sterilized and nonsterilized soil samples. The resulting seedlings were used as baits for the isolation of fungi. Seedlings growing on the natural, nonsterilized substrate were characterized by having a lower vegetative growth than the ones growing on the sterilized soil samples, and most of them died. *Phytophthora cinnamomi* was consistently isolated from their roots. *Fusarium oxysporum* was also isolated as well as different species of *Pythium*, although to a lesser extent. Pathogenicity tests were performed on holm oak seedlings with five different isolates of *P. cinnamomi*, with *F. oxysporum*, *Pythium* and with a mixture of the three fungi. All the inoculated seedlings with *P. cinnamomi* developed root rot and grew slowly, and 35.7% of them died up to the end of the experiments. *P. cinnamomi* was consistently isolated from their roots, indicating causal agent of holm oak decline. However, *F. oxysporum* caused similar symptoms on oak seedlings as *P. cinnamomi*, and was isolated also from the roots, although its frequency was lower than that of *P. cinnamomi*.

14 Effects of three fungal pathogens on water relations, chlorophyll fluorescence and growth of *Quercus suber* L.

Some physiological and growth parameters were studied in cork oak plants inoculated with three pathogenic fungi (*Botryosphaeria stevensii*, *Hypoxylon mediterraneum* and *Phytophthora cinnamomi*) in order to understand the effects of the fungal infection. All inoculated fungi induced the reduction of daily stem growth, stomatal conductance, air-leaf temperature and photochemical efficiency. The more aggressive pathogens, *B. stevensii* and *P. cinnamomi*, induced the sharpest decline in all growth and physiological parameters. A sudden decline of all variables was caused by *B. stevensii* within 6 days after inoculation, followed by a temporary increase in maximum daily shrinkage coinciding with witting of the plants. The plants inoculated with *P. cinnamomi* underwent a more gradual decrease in all parameters, whereas *H. mediterraneum* induced a slight but still significant reduction only in stem growth and shrinkage. Linear variable displacement transducers (LVDT) proved to be a suitable tool to measure some effects of the pathogenesis.

15 First record of *Phytophthora cinnamomi* on cork and holm oaks in France and evidence of pathogenicity

In 1995 and 1996, a survey for the presence of *Phytophthora cinnamomi* in cork and holm oak sites in southeastern France was carried out. Twenty-four sites were chosen. Tree decline severity and other characteristics were assessed. Subplots of four trees were more fully investigated: relative soil water content was assessed and *Phytophthora* isolation was attempted from soil samples. When cortical lesions were observed, isolations were carried out from infected tissues. In six cork oak and one holm oak sites, *P. cinnamomi* was isolated from soil or trunks. All the different isolates obtained in 1995 were aggressive on cork and holm oaks. However, these species were less susceptible than *Castanea sativa* and more susceptible than *Q. rubra*. These results confirm the pathogenicity of *P. cinnamomi* towards Mediterranean oaks and its possible involvement in the decline process of these species.

Artigo n.º	**Título**/Resumo
16	**Regulation by biotic stress of tannins biosynthesis in *Quercus ilex*: Crosstalk between defoliation and *Phytophthora cinnamomi* infection**

Sustainability of the Mediterranean forest is threatened by oak decline, a disease of holm oak and other *Quercus* species that is initiated by infection with the oomycete *Phytophthora cinnamomi*. Focusing on the role of tannins in the chemical defense of plants, this work investigated whether tannins content in *Quercus ilex* is regulated by biotic stress. Screening of published genomes allowed the identification of *Quercus* sequences encoding enzymes for early steps of the biosynthesis of phenolic compounds, like hydrolysable tannins and condensed tannins (CT) among others, plus genes involved in the late steps of CT biosynthesis. Four days after treatment of *Q. ilex* seedlings by mechanical defoliation, *P. cinnamomi* infection and both stressors simultaneously, mRNA concentrations for tannins biosynthesis enzymes were measured in leaves. Among the transcript amount for shikimate dehydrogenase (SDH, EC 1.1.1.25), anthocyanidin reductase (EC 1.3.1.77), anthocyanidin synthase (EC 1.14.11.19) and leucoanthocyanidine reductase (EC 1.17.1.3), defoliation induced gene expression for SDH2 isoenzyme. About 4 days after infection of roots by *P. cinnamomi*, this up-regulation was canceled and SDH enzyme activity decreased. Furthermore, during this late stage of biotrophic interaction the pathogen switched off the correlation engaged by defoliation between the expression of SDH1 and SDH2 encoding genes and chemical defenses corresponding to total tannins, which were down-regulated. Thus, tannins biosynthesis in seedlings of *Q. ilex* is induced after mechanical defoliation whereas infection by the pathogen interferes with this regulation, potentially increasing the susceptibility of plants to herbivory and aggravating the impact of biotic stress.

17	**The rhizosphere microbiome: A key component of sustainable cork oak forests in trouble**

Cork oak (*Quercus suber* L.) is an emblematic component of western Mediterranean basin landscapes. Cork oak forests have always been highly appreciated by local populations for their great ecological and socio economic value. However, the sustainability of cork oak forests has been threatened in recent decades by a complex phenomenon of degradation called "oak decline", worsened by the increase of anthropogenic and climatic disturbances combined with a lack or scarcity of natural cork oak regeneration. All these disturbances disrupt key components of the ecosystem, notably the rhizosphere microbiome, potentially sharpening decline processes and forest ecosystem degradation in return. This review provides an overview of the main abiotic and biotic decline drivers and a comprehensive state of knowledge about the impact of decline-related cork oak forest degradation on rhizosphere microbiome in Mediterranean basin. Finally, perspectives for the improvement of cork oak forest sustainability through the development of ecological management strategies based on rhizosphere microbiome-driven plant managements are highlighted.

18 Forecasting oak decline caused by *Phytophthora cinnamomi* in Andalusia: Identification of priority areas for intervention

Since the mid-20th century, trees in the Andalusian oak dehesa and forests have exhibited stress that often ends in the death of the tree. These events have been associated with *Phytophthora cinnamomi*, a soil borne root pathogen, which causes root rot, bark cankers, decay and mortality - known as oak decline. *Phytophthora cinnamomi* is most virulent under high ambient temperatures combined with moist soils, i.e., in Mediterranean areas. We used presence/absence point locations of the Andalusian Network for Damage Monitoring in Forest Ecosystems (RED SEDA) pathogen survey and four categories of environmental variables - meteorological, edaphic, topographic and tree cover - to accurately predict *Phytophthora cinnamomi* current and future potential distribution within Andalusia, for a range of climate change scenarios, using ensemble species distribution models (SDMs). We assessed which categories of environmental variables explained the distribution of the pathogen, obtained accurate predictions for the current potential distribution of *Phytophthora cinnamomi* (AUC > 0.95, TSS > 0.70, Kappa > 0.65) and forecasted its future potential distribution. Subsequently, we classified the sites of the pathogen survey within the RED SEDA network in three zones according to the already-recorded presence of the pathogen and the current and future predicted probability of occurrence. Finally, we suggested phytosanitary management strategies for each zone.

19 **Field susceptibility of cork oak trees with different provenances to *Phytophthora cinnamomi***

Cork oak decline has been a serious problem in Portuguese central and southern areas in the last 30 years. This decline is associated with *Phytophthora cinnamomi*, a pathogen with a major impact on cork oak decline. A programme aiming the plant selection on resistance, to address the infection of soilborne *P. cinnamomi* in cork oak woodlands, should therefore be fundamental for an integrated strategy for the management of the cork oak woodlands. In this context, the main objective of this study was to select potential tolerant families by evaluation of field susceptibility to the pathogen. During 10 years (2004-2014), the survival and height growth of 157 cork oak families from eight different provenances from Portugal and Spain were assessed in a field highly infested with *P. cinnamomi*. Results showed a high mortality of the plants, over the whole period, insofar that only 14 families, out of the 157, showed a survival rate between 40% and 60%. These families exhibited also a good height growth with heights ranging between 1 and 1.50m. It was also observed that ploughing, leading to better soil conditions, could contribute to improve the survival rate and the development of plants. Potential families of cork oak were identified as tolerant plant material and rootstock, thereby candidates to be used in restoration and reforestation. Interestingly, additional results concerning a *Quercus faginea* family evidenced a good resistance of this species to the pathogen. This information is indicative that *Q. faginea* could be used as a rootstock for the cork oak reforestation in areas where the pathogen had high impact.

20 **Early survival of *Quercus ilex* subspecies from diferent populations after infections and co-infections by multiple *Phytophthora* species**

Forests in Europe are threatened by increased diversity of *Phytophthora* species, but effects on trees of simultaneous infections by *Phytophthora* and ecological consequences of their coexistence are unknown. This study explored variation in early survival of *Quercus ilex* to *Phytophthora* infections and assessed interactions between *Phytophthora* species when trees were co-infected. Three *Phytophthora* species (*P. cinnamomi*, *P. gonapodyides* and *P. quercina*), seeds from 16 populations of *Q. ilex* (*ballota* and *ilex* subspecies) and two infection times were used as sources of variation in two experiments. The influence of *Phytophthora* species, *Q. ilex* subspecies and populations on plant germination and survival were analysed using generalized linear mixed models and survival analysis techniques. Germination rates were not influenced by *Phytophthora* spp. ($P = 0.194$) but by the subspecies and populations of *Q. ilex* ($P < 0.001$). In *Phytophthora*-infested soils, *Q. ilex* subsp. *Ilex* germinated at higher rates than *Q. ilex* subsp. *ballota*. Plant survival was strongly influenced by *Phytophthora* species ($P < 0.001$), not by the subspecies and populations of *Q. ilex*. Seedling mortality was reduced and delayed if a less virulent *Phytophthora* species infected plants prior to infection by a more virulent *Phytophthora* species. The results help to explain oak decline syndrome and the lack of natural and artificial regeneration of *Q. ilex* forests. Lack of interspecific variability of early survival to *Phytophthora* spp. discourages direct sowing for artificial reforestation programmes. Large, thick seeds, giving plants rapid growth, are advantageous traits when soils are infested with *Phytophthora* spp.

21 Differences in root growth of *Quercus ilex* and *Quercus suber* seedlings infected with *Phytophthora cinnamomi*

In the southwest of the Iberian Peninsula, *Phytophthora cinnamomi* Rands is causing irreversible damage to populations of the two most common species of *Quercus*, the holm oak (*Quercus ilex* L.) and the cork oak (*Quercus suber* L.). Although the symptoms are similar in the two species, the mortality rates are different. We found significant differences in the post-infection growth of the root system as a function of tree species, as well as initial plant size, and inoculum level. We observed a marked decrease in the growth of new roots in *Q. ilex* with increasing inoculum level, while in *Q. suber*, we found longer but thinner roots with a moderate inoculum level. In both species, we observed a worsening in the water status of the plants from the lowest inoculum level.

22 Seasonal variations of ectomycorrhizal communities in declining *Quercus ilex* forests: interactions with topography, tree health status and *Phytophthora cinnamomi* infections

Seasonal variations in ectomycorrhizal symbiosis of *Quercus ilex* trees were studied, with special emphasis on tree health status, *Phytophthora cinnamomi* root infections and topography. Five *Q. ilex* forests in western Spain were selected, and ca. 40 000 root tips from 3 declining and 3 non-declining trees per forest were examined. Ectomycorrhizal fungal abundance and *P. cinnamomi* root infections were quantified seasonally for 2 years, and an assessment was made of the water soil content and physiological status of trees during summer, the most stressful season. Seasonal changes in ectomycorrhizal fungal abundance were higher for *Tomentella* spp. than those for *Russula* spp. and *Cenococcum geophilum*, but these changes were not influenced by topography, tree decline status and *P. cinnamomi* root infections. Seasonal variations of nonvital tips were conditioned significantly by the decline status of trees. A higher proportion of non-vital tips and a lower proportion of vital nonmycorrhizal tips were observed in declining than in non declining trees. Abundance of *C. geophilum* and *Tomentella* spp. was not influenced by *P. cinnamomi* root infections but by topography. Abundance of *Russula* spp. was not influenced by *P. cinnamomi* root infections but by the decline status of trees. The relationships found between tree physiology and ectomycorrhizal abundance changed significantly depending on the decline status of trees. For the first time, seasonal variations in ectomycorrhizae in *P. cinnamomi*-infected *Q. ilex* forests are reported. The low diversity and abundance of ectomycorrhizal fungi associated with declining trees should be further studied in order to uncover causes or consequences of *Q. ilex* crown transparency.

23 Induction of defence responses by cinnamomins against *Phytophthora cinnamomi* in *Quercus suber* and *Quercus ilex* subs. *rotundifolia*

The strong association between *Phytophthora cinnamomi* and the mortality and decline of *Quercus suber* and *Q. ilex* subsp. *rotundifolia* has been known for two decades. The ability of elicitins secreted by this pathogen to trigger defence responses in these *Quercus* against itself was evaluated in this work. Biomass quantification by quantitative real-time PCR revealed a significant decrease in pathogen colonization of *Q. suber* roots after 24 h pre-treatment with alpha- and beta-cinnamomin. In *Q. suber* and *Q. ilex* roots pre treated with alpha-cinnamomin, hyphae were unable to reach and colonize the vascular cylinder and showed cytoplasmic disorganization in all the roots observed as contrasted with non-pretreated roots. The pathogen was restricted to the intercellular spaces of the cortical parenchyma and the concomitante accumulation of electron dense materials was observed in contact with the hyphae. Furthermore, ROS (reactive oxygen species) production and the enzymatic activities of superoxide dismutase, catalase and peroxidase were compared in infected and non-infected *Quercus* roots in time course trials. There was a significant increase in the production of hydrogen peroxide (H_2O_2) and superoxide anion (O^{2-}) and an enhanced activity of the enzymes in infected roots was observed at each time point. When comparing with elicitin non-treated roots, the alpha-cinnamomin-treated roots in interaction with *P. cinnamomi* showed a decrease in ROS accumulation and an increase of the enzyme activities. The overall results were consistent with an induction by the cinnamomins which initiated defence responses against the pathogen invasion of roots. Finally, elicitins were immunolocalized in the contact zone of *P. cinnamomi* hyphae with epidermal host cells, plasmalemma outer cytoplasm and around the intracelular hyphae in the vacuoles of invaded epidermal cells.

24 Histology of *Quercus ilex* roots during infection by *Phytophthora cinnamomi*

The speed of infection of *Quercus ilex* by *Phytophthora cinnamomi* is influenced by the method of inoculation used, and structural changes in the host do not differ depending on whether primary or secondary roots are infected. This study aimed to elucidate the infection process of the invasive pathogen *Phytophthora cinnamomi* on primary and secondary roots of 2-month old *Quercus ilex* seedlings. To test if different methods of inoculation lead to different changes in the host caused by the pathogen, the root system of plants was either immersed into a suspension of *P. cinnamomi* zoospores, or placed in direct contact with agar plugs colonized by *P. cinnamomi* mycelium. Histology of root sections obtained every 24 h for 10 days revealed similar changes in the structure of cells and tissues of the host irrespective of the inoculation method used. However, the immersion method resulted in a delay in the colonization of the host, different aerial symptoms, and the formation of different reproductive structures of the pathogen. Emerging secondary and tertiary roots and sites where secondary or tertiary roots were about to emerge were identified as main entry points. Hyphae in the xylem tissues were more frequently found in secondary than in primary roots, but in both types of roots the phloem was the most important pathway of colonization. For the first time in the interaction between *Q. ilex* and *P. cinnamomi*, transmission electron microscopy was used to describe degradation of the host cell walls, pit penetration and extrahaustorial matrix. Haustoria development during intracellular growth and hyphal aggregations (stromata) caused no damage to the host cell walls indicating hemibiotrophic parasitism.

25 Histopathology of infection and colonization of *Quercus ilex* fine roots by *Phytophthora cinnamomi*

Quercus ilex is one of the European forest species most susceptible to root rot caused by the oomycete *Phytophthora cinnamomi*. This disease contributes to holm oak decline, a particularly serious problem in the dehesas ecosystem of the southwestern Iberian Peninsula. This work describes the host-pathogen interaction of *Q. ilex* and *P. cinnamomi*, using new infection indices at the tissue level. Fine roots of 6-month old saplings inoculated with *P. cinnamomi* were examined by light microscopy and a random pool of images was analysed in order to calculate different indices based on the measured area of pathogen structures. In the early stages of invasion, *P. cinnamomi* colonizes the apoplast and penetrates cortical cells with somatic structures. On reaching the parenchymatous tissues of the central cylinder, the pathogen develops different reproductive and survival structures inside the cells and then expands through the vascular system of the root. Some host responses were identified, such as cell wall thickening, accumulation of phenolic compounds in the middle lamella of sclerenchyma tissues, and mucilage secretion blocking vascular cells. New insights into the behaviour of *P. cinnamomi* inside fine roots are described. Host responses fail due to rapid expansion of the pathogen and a change in its behaviour from biotrophic to necrotrophic.

26 Ectomycorrhizal symbiosis in declining and non-declining *Quercus ilex* trees infected with or free of *Phytophthora cinnamomi*

Quercus ilex decline and the presence of the soil-borne pathogen *Phytophthora cinnamomi* are hypothesised to be associated with shifts in ectomycorrhizal fungi abundance. Soil properties may also influence the relation of this pathogen to ectomycorrhizae. To investigate these associations, 96 *Q. ilex* declining stands were selected in western Spain and both declining and non-declining trees were sampled. Soil properties (soil depth, Ah horizon thickness, texture, pH, redox potential, soil bulk density and N-NH^{4+} and N-NO^{3-} concentrations), *P. cinnamomi* root infections and ectomycorrhizal fungi abundance were assessed. The most dominant ectomycorrhizal morphotypes were *Cenococcum geophilum*, *Tomentella* spp. and *Russula* spp. Lower percentages of non-vital and vital ectomycorrhizal root tips were observed in declining than in nondeclining trees. No significant differences in parameters characterising the ectomycorrhizal community between trees infected with or free of *P. cinnamomi* were observed. However, results indicate that non-mycorrhizal root tips are vulnerable entry points for the pathogen into the tree. More tips were colonised by *Russula* spp. and other ectomycorrhizal fungi in non-declining infected trees than in declining infected trees. Trees growing on stream banks showed a lower abundance of ectomycorrhizal root tips in fine-textured soils than in coarse-textured soils. Ectomycorrhizal tip abundance was positively related to Ah horizon thickness, irrespective of tree health status. The presence of *P. cinnamomi* altered relations between ectomycorrhizal abundance and several soil properties. The limited ectomycorrhizal diversity in *Q. ilex* declining stands and interactions of ectomycorrhizal abundance with soil texture, topography and *P. cinnamomi* root infections are reported and discussed.

27 **Cryptogein and capsicein promote defence responses in *Quercus suber* against *Phytophthora cinnamomi* infection**

The decline of cork oak (*Quercus suber*) stands in Iberian Peninsula is associated with infection by *Phytophthora cinnamomi*. Most *Phytophthora* species secrete elicitins, which can enhance defence reactions against some pathogens. Here cytological and physiological effects of the elicitins cryptogein and capsicein on cork oak root infection by *P. cinnamomi* were evaluated. The progression of the pathogen in root tissue and its effects on total fatty acid (TFA) composition of roots and leaves were analysed in seedlings. Net photosynthesis (P_n), stomatal conductance (g_s), chlorophyll a fluorescence measurements (quantum yield of linear electron transport ϕ_e, photochemical quenching q_P non-photochemical quenching NPQ) and carotenoid determinations were carried out in well established (4 months) plants. In elicitin-treated roots, 2 days after inoculation, the pathogen which presented loss of viability and membrane degradation was mainly restricted to the intercellular spaces of the cortical parenchyma, and did not reach the vascular cylinder. Electron dense materials accumulated in the intercellular spaces of the cortex next to disorganized hyphae, suggested to be related with defence reactions. Cryptogein (or its interaction with *P. cinnamomi*) induced enhanced lipid synthesis in leaves, which may contribute to preserve membrane stability. *P. cinnamomi* decreased P_n, g_s, ϕ_e, and q_P, whereas elicitin-treated plants displayed values similar to controls. Overall, the results indicated a resistance response of cork oak against this oomycete, induced by the elicitins.

28 **Susceptibility to *Phytophthora cinnamomi* of the commonest morphotypes of Holm oak in southern Spain**

The four main morphotypes of Holm oak (*Quercus ilex* subsp. *ballota*) present in Andalusia (*expansa*, *macrocarpa*, *microcarpa* and *rotundifolia*) were infected with

Phytophthora cinnamomi to determine their susceptibility to the root pathogen. No large differences were found among the four morphotypes in the infection of roots, which always showed a high degree of necrosis. However, the different responses of the foliage to infection separated the four morphotypes of Holm oak into three groups: very susceptible (*microcarpa*), susceptible (*expansa*) and moderately susceptible (*rotundifolia* and *macrocarpa*). The natural hybrid *Q. ilex ballota – Q. faginea* exhibited a low level of root and foliar symptoms when infected with *P. cinnamomi*. *Quercus faginea* could be considered as a source of resistance to *P. cinnamomi* in future breeding programes.

29 Involvement of the β-cinnamomin elicitin in infection and colonisation of cork oak roots by *Phytophthora cinnamomi*

The virulence of two wild type (PA45 and PA37) and two genetically modified (13C: hygromycin resistant; FATSS: hygromycin resistant and beta-cin knock-down) *Phytophthora* cinnamomi *strains* towards cork oak (*Quercus suber*) was assessed via a quantitative evaluation of disease symptoms arising from a soil infestation assay, and by a histological analysis of root colonization. Comparison of virulence, as expressed by symptom severity, resulted in the following ranking: highly virulent (wild type strains), medium virulence (strain 13C) and weakly virulent (FATSS). Both transgenic strains were compromised in their virulence, as expressed by symptom severity, but strain 13C was much less affected than FATSS. Microscopic observation showed that the FATSS strain was unable to effectively invade the root, while 13C and the two wild type strains were all able to rapidly colonize the whole root, including the vascular tissue. These results strengthen the notion that elicitins are associated, either directly or indirectly, with the infection process of *Phytophthora*.

30 Susceptibility of Iberian trees to *Phytophthora ramorum* and *P. cinnamomi*

The capacity of *Phytophthora ramorum* to colonize the inner bark of 18 native and two exotic tree species from the Iberian Peninsula was tested. Living logs were wound-inoculated in a growth chamber with three isolates belonging to the EU1 and two to the NA1 clonal lineages of *P. ramorum*. Most of the *Quercus* species ranked as highly susceptible in experiments carried out in summer, with mean lesion areas over 100 cm^2 in *Q. pubescens*, *Q. pyrenaica*, *Q. faginea* and *Q. suber* and as large as 273 cm^2 in *Q. canariensis*, ca. 40 days after inoculation. *Quercus ilex* ranked as moderately susceptible to *P. ramorum*, forming lesions up to 133 cm^2 (average 17.2 cm^2). *Pinus halepensis* and *P. pinea* were highly susceptible, exhibiting long, narrow lesions; but three other pine species, *P. pinaster*, *P. nigra* and *P. sylvestris*, were resistant to slightly susceptible. No significant difference in aggressiveness was found between the isolates of *P. ramorum*. In addition, there was evidence of genetic variation in susceptibility within host populations, and of significant seasonal variation in host susceptibility in some *Quercus* species. The results suggest a high risk of some Iberian oaks to *P. ramorum*, especially in forest ecosystems in southwestern Spain, where relict populations of *Q. canariensis* grow amongst susceptible understory species such as *Rhododendron* ponticum and *Viburnum tinus*. One isolate of *P. cinnamomi* used as positive control in all the inoculations was also highly aggressive to Iberian oaks and *Eucalyptus dalrympleana.*

31 *Quercus suber* Infected by *Phytophthora cinnamomi*. Effects at cellular level of cinnamomin on roots, stem and leaves

Phytophthora cinnamomi has been reported to be regularly associated with cork and holm oak decline. This oomycete secretes elicitins, a group of unique highly conserved proteins that can enhance plant defence reactions. In our previous work it was shown that the absorption of one of these elicitins– α-CIN– by the roots of cork and holm oak at concentrations of 500 µg and 1 mg/ml reduced *P. cinnamomi* viability and its progression into internal tissues. The objectives of the present work were to study the restriction of host tissue colonization and the loss of pathogen viability in the roots treated with lower concentration of α -CIN, and to verify structural alterations at cellular level induced by this elicitin in aerial part of the plantlets. Roots of *Quercus suber* seedlings two month old were immersed in aqueous α-CIN solutions [250 µg/ml] and [100 µg/ml] during 24 h. Roots were then inoculated with *P. cinnamomi.* The samples pre-treated with α-CIN at the two concentrations and inoculated with *P. cinnamoni* revealed similar histological and cytological aspects. The hyphae were mainly intercellularly located, restricted to the external cortex. The treatment induced loss of pathogen viability and it was evident the pathogen membranar degradation, loss of organelles and reduction of cytoplasm density. The hyphae were mainly intercellularly. In stem of α-CIN pre-treated plants, the external cortical and medullar parenchymas concentrated osmiophylic materials in vacuoles. In leaves the histological structure was maintained, but in spongy parenchyma the chloroplast ultrastructure was modified. In leaves the histological structure was maintained, but in spongy parenchyma the chloroplast ultrastructure was modified. The loss of viability of the pathogen and its confinement to the root cortex are in accordance with our previous observations as well as those by other authors. The increase of accumulation of osmiophylic materials, probably phenol compounds, in the stem suggests a defence response. In leaves the chloroplasts are the most sensitive organelles but did not appear much damaged, allowing the recovering of the plantlets. We can conclude that α-CIN induced a defence response against *P. cinnamomi* in cork oak, at 100 µg/ml and no irreversible damage occurred.

32 Seasonal and spatial mortality patterns of holm oak seedlings in a reforested soil infected with *Phytophthora cinnamomi*

The viability of 1-year-old holm oak (*Quercus ilex*) seedlings in a soil naturally infected with *Phytophthora cinnamomi* was studied during 2 consecutive years in a plot located in south-western Spain. In both years, total mortality during autumn and winter was not noticeable (<2.1%). In spring, mortality levels were higher (8.3-4.6%), especially the first year. A steep increase in total mortality occurred in summer, both in the first (11.4%) and second (24.2%) year, but mortality attributable to *P. cinnamomi* was 1.9 and 7.6%, respectively. Thus, 2 years after planting, total cumulative mortality was 43.4%, and that attributable to *P. cinnamomi* 9.6% (i.e. 22.1% of total mortality). Fungus-derived mortality followed a spatially aggregated pattern in the reforestation plot, suggesting a clustered distribution of the inoculum in the soil. Furthermore, mortality by *P. cinnamomi* was also associated with nearness of infected adult trees in the plot. Results obtained are discussed in the framework of seasonal water deficit, *P. cinnamomi* damage, weed competition and sanitation techniques to be used in declined holm oak stands in Spain.

33 Viability of holm and cork oak seedlings from acorns sown in soils naturally infected with *Phytophthora cinnamomi*

The emergence and survival of pregerminated holm oak (*Quercus ilex*) and cork oak (*Quercus suber*) acorns from two ecologically different dehesas (Mediterranean open woodlands) were studied in two soils from these stands naturally infected with *Phytophthora cinnamomi*, and in the same soils previously sterilized in the autoclave.

Phytophthora cinnamomi was consistently isolated from the radicles of all unemerged and all emerged but dead seedlings from the unsterilized substrates. Seedlings of holm oak were more susceptible to *P. cinnamomi* than those of cork oak. Mortality of holm oak seedlings was significantly different depending only on soil treatment (sterilized or unsterilized), and it was 100% in unsterilized soils, independent of acorn provenance and soil origin. Mortality of cork oak seedlings was significantly different depending on the acorn origin and soil treatment, and on the interactions acorn origin x soil origin and soil origin x soil treatment. The demonstrated high susceptibility of holm and cork oak young seedlings to *P. cinnamomi* could be a limiting factor in Mediterranean open woodlands (*dehesas*) not only in natural regeneration processes but also when reforestation by direct sowing is implemented.

34 Effects of root damage associated with *Phytophthora cinnamomi* on water relations, biomass accumulation, mineral nutrition and vulnerability to water deficit of five oak and chestnut species

The effects of root damage associated with *Phytophthora cinnamomi* on water relations, biomass accumulation, mineral nutrition and vulnerability to water deficit were investigated in pedunculate oak (*Quercus robur*), red oak (*Quercus rubra*) and holm oak (*Quercus ilex*) saplings over two years. Comparison was made with sweet chestnut (*Castanea sativa*), a susceptible species to infection by *P. cinnamomi*, and with a resistant hybrid chestnut (*Castanea crenata* x *C. sativa*). Trees were inoculated in 1998 and were subjected to water shortage in 1999. All inoculated sweet chestnuts died before the application of water shortage. Hybrid chestnut, pedunculate oak and red oak displayed low root susceptibility to *P. cinnamomi*. In these species, water relations, aerial growth and mineral nutrition were slightly affected by inoculation. By contrast, holm oak was the most susceptible oak species to *P. cinnamomi* as inoculated well-watered trees displayed the highest root loss (67%) and 10% mortality. Root loss was associated with a decrease in predawn leaf water potential, a 61% reduction in stomatal conductance, a 55% reduction in aerial biomass, a decrease in leaf carbon isotope discrimination and reduced leaf N and P contents in comparison with controls. In hybrid chestnut and pedunculate oak, water shortage resulted in a similar decrease of predawn leaf water potential, stomatal conductance and aerial biomass in inoculated and non-inoculated trees. In red and holm oaks, soil volumetric water content of inoculated trees subjected to water shortage remained high. The effects observed in those trees were similar to those of inoculated well-watered trees and were probably the result of root infection only.

35 Root infection by *Phytophthora cinnamomi* in seedlings of three oak species

The combination of soil infestation with *Phytophthora cinnamomi* and repetitive flooding was studied on 1 year-old plants of *Quercus ilex* (holm oak) and *Q. suber* (cork oak). In a second experiment, using 2-year old plants of the same species and of red oak (*Q. rubra*), the soil infestation was followed by two drought rewatering cycles. Oak predawn leaf water potential (PLWP) and stomatal conductance (gs) were monitored during both experiments. Root infection, root loss, wilting and mortality were assessed at the end of the experiments. *Q. ilex* exhibited the highest susceptibility to *P. cinnamomi*, and *Q. rubra* the lowest. Root infections caused by *P. cinnamomi* were more severe in the flooding than in the drought experiment. The most noticeable effect of the infection on plant water relations was a decrease in stomatal conductance. This occurred at different times after inoculation, varying with species susceptibility and experiment. Inoculation with *P.* cinnamomi induced a decrease of PLWP in *Q. ilex* plants, and in some *Q. suber* plants exhibiting a severe root loss. The results further showed that the relationship between PLWP and gs was modified by infection with *P. cinnamomi*. The combination of flooding and infection with *P. cinnamomi* acted synergistically on the water relations of *Q. ilex*. By contrast, there was no significant increase in disease severity due to the postinoculation water stress imposed on the oaks.

36 Evidence for *Phytophthora cinnamomi* involvement in Iberian oak decline

Rapid and sometimes extensive mortality and decline of oak, principally *Quercus suber* and *Q. ilex*, has occurred in parts of Southern Spain and Portugal in recent decades. We report here isolation of the aggressive root pathogen *Phytophthora cinnamomi* from roots of diseased oaks or from soil at eleven out of thirteen decline foci examined. It is proposed that the introduction and spread of *P. cinnamomi* may be a major factor in the Iberian oak decline, interacting with drought and other site factors, and leading to stress related attacks by insects and other fungi. By analogy, it may also be involved in the similar oak declines occurring elsewhere on the Mediterranean.

Artigo n.º **Título**/Resumo

37 **Differences in the response to acute drought and *Phytophthora cinnamomi* Rands infection in *Quercus ilex* L. seedlings**

The sustainability of dehesas is threatened by the holm oak decline. It is thought that the effects of root rot on plant physiology vary depending on external stress factors. Plant growth and biomass allocation are useful tools to characterize differences in the response to drought and infection. The study of physiological responses together with growth patterns will clarify how and to what extent root rot is able to damage the plant. A fully factorial experiment, including drought and *Phytophtora cinnamomi* Rands infection as factors, was carried out with *Quercus ilex* L. seedlings. Photosynthesis, biomass allocation and root traits were assessed. Photosynthetic variables responded differently to drought and infection over time. The root mass fraction showed a significant reduction due to infection. *P. cinnamomi* root rot altered the growth patterns. Plants could not recover from the physiological effects of infection only when the root rot coincided with water stress. Without additional stressors, the strategy of our seedlings in the face of root rot was to reduce the biomass increment and reallocate resources. Underlying mechanisms involved in plant-pathogen interactions should be considered in the study of holm oak decline, beyond the consideration of water stress as the primary cause of tree mortality.

38 **Environmental factors associated with the spatial distribution of invasive plant pathogens in the Iberian Peninsula: The case of *Phytophthora cinnamomi* Rands**

Although spatial variability in the distribution of soil organisms is often regarded as random, recent attempts have found significant spatial structure at several scales. Understanding the drivers of this variability at large scale could help to evaluate the ecological and socio-economic impacts of soil organisms in the ecosystems. In the present study we aim to (i) understand the relative role of environmental factors and human influence on the distribution of one of the World's most destructive and invasive plant pathogens, *Phytophthora cinnamomi* Rands, and (ii) to estimate its potential geographic distribution in the Iberian Peninsula as a proxy of its potential impact. For this purpose, we used a total of 277 records of *P. cinnamomi* (presence: n = 157;

absences: n = 120) in the Iberian Peninsula and three sets of explanatory variables reflecting abiotic conditions (climate and soil), biotic conditions (main susceptible host tress distribution and vegetation cover based on NDVI) and human influence. The current distribution of *P. cinnamomi* in the Iberian Peninsula seems to be influenced principally by fine texture soil and climate, following the land use and lastly the presence of its main host forest species. Its potential distribution across the Iberian Peninsula suggests potential for further expansion along the northeast and southeast of Spain and central Portugal. Given the significant impact of this pathogen on forest ecosystems, the modelling of its distribution in the Iberian Peninsula offers an important decision tool for the monitoring and restoration of declining Mediterranean oak forests.

39 Influence of temperature on germination of *Quercus ilex* in *Phytophthora cinnamomi*, *P. gonapodyides*, *P. quercina* and *P. psychrophila* infested soils

The influence of temperature on germination of *Quercus ilex* acorns in *Phytophthora* infested soils was quantified for the first time. Radicle damage and mortality of *Q. ilex* seeds germinating at 17, 20, 23 and 26°C in *Phytophthora cinnamomi*, *P. gonapodyides*, *P. quercina* and *P. psychrophila* infested soils were assessed and related to *in vitro* mycelium growth of the same isolates of the pathogens. The optimum growth temperatures of isolates of *P. cinnamomi*, *P. gonapodyides*, *P. quercina* and *P. psychrophila* were 20-23, 23-26, 20-23 and 20°C, respectively. At 17 and 20°C, all four *Phytophthora* species caused 100% acorn mortality, whereas at 26°C, acorn mortality was 100, 10, 25 and 0% in *P. cinnamomi*, *P. gonapodyides*, *P. quercina* and *P. psychrophila* infested soils, respectively. At 23°C, *P. cinnamomi* and *P. gonapodyides* reduced acorn radicle length more than *P. quercina* and *P. psychrophila*, whereas at 26°C, only *P. cinnamomi* caused further reduction in radicle length. The higher susceptibility of germinating acorns in comparison to seedlings reported in the literature indicates age-related susceptibility of *Q. ilex* to *Phytophthora*. The seedling/pathogen growth ratio was inversely related to the reduction in radicle length at different temperatures (R^2_{Adj} = 0.84, p<0.0001), suggesting that rapid germination may allow seedlings to escape from infection. Increasing temperatures had different effects on damage to acorns depending on the pathogen present in the soil, indicating that *Phytophthora* species x temperature interactions determined *Q. ilex* germination. The effects of temperature on the impacts of *Phytophthora* species based on climate change predictions for Mediterranean countries are discussed.

40 **Drought events determine performance of *Quercus ilex* seedlings and and increase their susceptibility to *Phytophthora cinnamomi***

More frequent weather extremes are expected to occur in the Mediterranean region within the present context of climate change. These extremes could affect forests and plant diseases driven by pathogens. It is hypothesised that simulation of weather extremes during *Quercus ilex* growth will influence early performance and susceptibility to the invasive oomycete *Phytophthora cinnamomi*. In 2010, 140 *Q. ilex* seedlings were subjected to three watering regimes under greenhouse conditions: waterlogging (W), water stress (S) and optimal watering regime for growth (C). During the second vegetative period, conditions were altered to create the following scenarios: WW, WS, SS, SW and CC. After the second vegetative period, plants were artificially infested with *P. cinnamomi*. Holm oak (*Q. ilex*) was more sensitive to flooding in the first year of growth than in the second year. The altered scenarios produced plants with a lower fine-to-total root ratio than plants in unaltered scenarios. Plants with the highest growth rates maintained their relatively rapid growth and photosynthetic activity under altered scenarios. However, plants with the highest growth rates became the plants with the lowest growth rates when two consecutive years of drought occurred, indicating a trade-off by *Q. ilex* in growth investment, observed only if the water stress scenario persists. Seedlings were more sensitive to water shortage than to waterlogging, especially if they encountered a dry scenario during the first year. Exposure to drought events increased seedling mortality rates after *P. cinnamomi* infection. Waterlogging combined with subsequent water deprivation was the worst scenario when soil was infested with *P. cinnamomi*, causing 100% mortality of plants.

41 ***Quercus ilex* forests are influenced by annual variations in water table, soil water deficit and fine root loss caused by *Phytophthora cinnamomi***

It is hypothesised that major reductions in tree vitality are related to marked changes in soil water content, extremely wet winters followed by dry summers, and the presence of pathogenic organisms which take advantage of this situation. This study helps clarify the role of anual variations in water table, soil water content and fine root abundance in the decline of *Quercus ilex* forests, with special focus on trees growing in *Phytophthora cinnamomi*-infested soils. Conducted in western Spain, the study included 5 *Q. ilex dehesa* stands in which tree water status and soil water dynamic were compared in declining and non-declining trees, and 96 additional stands in which fine root abundance and pathogen assessment were compared in declining and non-declining trees. Declining trees showed significantly lower values than non-declining trees for leaf water potential and stomatal conductance. The period of waterlogging (2 months maximum, fluctuating from 0.5 to - 4.5 m), the soil water content values observed in summer (significantly higher in declining trees) and the similar presence of *Pythium spiculum* in declining and non-declining trees are not sufficient in themselves to explain tree health status. However, fine root density was 16.2% lower in declining than non-declining trees and 42% lower in *P. cinnamomi*-infected than non-infected trees. Root damage caused by *P. cinnamomi* in combination with periods of saturated soils favourable for the pathogen but unfavourable for the tree, coupled with small-scale differences in soil water-holding capacity, explain the symptoms and water status of declining trees. The combination of root damage and water stress explained above-ground symptoms of declining trees and will probably determine tree survival.

42 Influence of site factors on the impact of *Phytophthora cinnamomi* in cork oak stands in Portugal

Although decline of cork (*Quercus suber*) and holm oak trees (*Quercus rotundifolia*) has been described in Portugal in the late years of the 19th century, its development has become a motive of high concern during the last two decades. The presence of *Phytophthora cinnamomi* in cork and holm oak stands was surveyed in four different regions of the country (Trás-os-Montes, Alentejo, Ribatejo and Algarve) during 1995-98. Tree decline severity, sudden death and site characteristics were assessed in 56 sites representing varied conditions. The pathogen was isolated from oak roots and rhizosphere samples in 27 of those places. Other plant species from natural vegetation were sampled in three active disease centres. This survey showed that 56% of the surveyed species of shrub flora were infected with *P. cinnamomi*, which was detected mainly on species belonging to the families *Ericaceae*, *Cistaceae* and *Leguminosae*. Recovery of *P. cinnamomi* was more frequent in shallow soils (Leptosols and complex Leptosols and Luvisols). These soils are more common in the south (Algarve), where decline has a high impact. Soils with low fertility and low mineral nutrient levels, particularly phosphorus, seemed to favour infection. Site aspect and topographic tree situation were also evaluated. Sites facing south showed higher occurrence of *P.*

cinnamomi, which was also more frequent in slopes and valleys than on hilltops. In Algarve, a relationship could be established between the crown status and the presence of *P. cinnamomi* in roots and rhizosphere. Different morphotypes of *P. cinnamomi* could be distinguished *in vitro*, and their occurrence in the field was correlated with particular site characteristics. Further research needs and management strategies to limit the extension of the disease are discussed.

43 **Synthesis of dehydrin-like proteins in *Quercus ilex* L. and *Quercus cerris* L. seedlings subjected to water stress and infection with *Phytophthora cinnamomi***

The synthesis of dehydrin-like proteins in 24-month-old *Quercus cerris* and *Q. ilex* seedlings subjected to water stress and/or infection with *Phytophthora cinnamomi*, was investigated. An estimated 50 kDa dehydrin-like protein was observed in leaves of symptomless *Q. ilex* (drought tolerant) in control, as well as in seedlings infected with *P. cinnamomi*, throughout the experiment. Water stress, with a midday stem water potential value lower than - 2.7 MPa (up to 8 weeks), increased band intensity. Two new polypeptides with a molecular weight of about 60 and 63 kDa, respectively, were detected in *Q. ilex*, under enhanced water stress conditions ($\leq$ –4.0 MPa). No dehydrin-like proteins were observed in leaves of symptomless *Q. cerris* (drought susceptible) neither in control nor in infected treatments. With midday stem water potential lower than -3.8 MPa (up to 7 weeks), water stress induced the synthesis of a protein with an approx. molecular weight of 43 kDa, while a band of about 36.5 kDa was detected for both water stress and infection treatments. These results suggest the involvement of dehydrin-like proteins in the response of *Q. ilex* and *Q. cerris* seedlings to different stresses.

44 **Seasonal changes in susceptibility of *Quercus suber, Botryosphaeria stevensii* and *Phytophthora cinnamomi***

Monthly inoculations of both intact plants and excised shoots of *Quercus suber* with the pathogenic species *Botryosphaeria stevensii* and *Phytophthora cinnamom*i *were* performed to investigate seasonal changes in susceptibility of this forest tree species in relation to environmental parameters and plant water status. Infection symptoms were mainly detected on seedlings inoculated from spring to autumn (April through October) with either pathogen. Mean canker sizes also showed a seasonal pattern, the higher values being recorded in the same period as above. Lesion lengths were significantly ($P < 0.001$) related to environmental minimum temperature. Mean daily minimum temperatures within the range of 5-12°C clearly inhibited lesion development of *P. cinnamomi*, whereas *B. stevensii* showed a less pronounced decrease in canker expansion at the same temperature range. In excised shoots of *Q. suber* inoculated monthly with *B. stevensii*, a negative linear relationship was found between the studied range of plant relative water content (81-91 %) and canker length. In contrast, the lesions caused by *P. cinnamomi* were not significantly ($P = 0.32$) related to any seasonal change in water content. Some control measures for the diseases caused by both pathogens are discussed on the basis of the seasonal changes in host susceptibility observed in this study.

45 ***Phytophthora cinnamomi*** **and oak decline in southern Europe. Environmental constraints including climate change**

One of the most destructive of all tree root pathogens, the oomycete fungus *Phytophthora cinnamomi*, is associated with mortality and decline of *Quercus suber* and *Q. ilex* in the Mediterranean region. The symptoms and distribution of this decline are described. P cinnamomi is a primary pathogen on a very wide range of trees and woody ornamentals worldwide, but is probably a native of the Papua New Guinea region. It is soil borne and requires warm, wet soils to infect roots. Since 1900 it has caused major epidemics on native chestnuts in the United States and Europe, and now threatens the stability of entire forest and heath communities ecosystems in some parts of Australia. Together with drought, ii may be a major predisposing factor in the Iberian oak decline. Its possible role in this decline including its interaction with drought is discussed, and a generalised working hypothesis of decline is presented. The potential influence of climate warming on the activity of *P. cinnamomi*, is also considered. A model based on the CLIMEX program suggests that warming would significantly enhance the activity of the pathogen at its existing disease locations (such as the western Mediterranean and coastal northwest Europe), but that it would not greatly extend its activity into areas with cold winters such as central and eastern Europe.

Artigo n.º **Título**/Resumo

46 **Assessment of functional and structural changes of soil fungal and oomycete communities in holm oak declined dehesas through metabarcoding analysis**

Forest decline is nowadays a major challenge for ecosystem sustainability. *Dehesas*, which consists of savannah-like mediterranean ecosystems, are threatened by the holm oak decline in the south-west of Iberian Peninsula. *Phytophthora cinnamomi* is considered the main agent of holm oak root rot, but little is known about the relationship between diversity of soilborne microbial community and the decline syndrome of holm oak. It would be hypothesized that the changes in the structure and functionality of the soil microbiome might influence tree health status through changes in richness and diversity of beneficial organisms such as mycorrhizal species, or fungal plant pathogens such as *Fusarium* spp. or *Alternaria* spp. Total DNA of soil samples from declined oak *dehesas* was extracted and analyzed through metabarcoding techniques, to evaluate the specific composition and diversity of the fungal and oomycete communities and their relationship with the disease symptoms. The fungal community included a wide range of pathogens and abundance of ectomycorrhizal key taxa related with low defoliation degree. *Phytophthora cinnamomi* and *Pythium spiculum* did not appear among the most abundant oomycetes, nor were they related directly to defoliation levels. Moreover, a particular taxon belonging to the genus *Trichoderma* was strongly correlated with the scarcity of pathogenic *Phytophthora* spp. The diversity and composition of fungal and oomycete communities were related to the severity of the decline symptoms. The metabarcoding study of microbiome represents a powerful tool to develop biocontrol strategies for the management of the holm oak root rot.

47 **Conservation of holm oak (*Quercus ilex*) by *in vitro* culture**

In vitro culture techniques are used to propagate tree species, as well as to conserve the species in the short and long term. In the present study, *in vitro* propagation and conservation of holm oak (*Quercus ilex* L.) were successfully achieved using juvenile material. Mature acorns were germinated under controlled conditions of moisture and temperature, and 3-month-old seedlings were used as source of explants for culture initiation. Micropropagation via axillary bud proliferation was achieved by culturing shoots in a vertical position on Woody Plant Medium containing different cytokinins and/or concentrations, which were changed every 2 weeks over a 6-week multiplication cycle, as follows: 0.1 mg L^{-1} benzyladenine (BA) for the first 2 weeks, 0.05 mg L^{-1} BA for the next 2 weeks, and 0.01 mg L^{-1} BA plus 0.1 mg L^{-1} zeatin for the last 2 weeks. Acceptable rooting rates were obtained by culturing microcuttings in Murashige & Skoog medium with half-strength macronutrients supplemented with 3 or 5 mg L^{-1} indole-3-butyric acid (IBA) in combination with 0.1 mg L^{-1} naphthalene acetic acid (NAA) for 15 days and subsequent transfer to auxin-free medium for 4 weeks.

48 **De novo assembly of *Phlomis purpurea* after challenging with *Phytophthora cinnamomi***

Background: *Phlomis* plants are a source of biological active substances with potential applications in the control of phytopathogens. *Phlomis purpurea* (Lamiaceae) is autochthonous of southern Iberian Peninsula and Morocco and was found to be resistant to *Phytophthora cinnamomi*. *Phlomis purpurea* has revealed antagonistic effect in the rhizosphere of *Quercus suber* and *Q. ilex* against *P. cinnamomi*. *Phlomis purpurea* roots produce bioactive compounds exhibiting antitumor and anti-Phytophthora activities with potential to protect susceptible plants. Although these important capacities of *P. purpurea* have been demonstrated, there is no transcriptomic or genomic information available in public databases that could bring insights on the genes underlying this anti-oomycete activity. Results: Using Illumina technology we obtained a de novo assembly of *P. purpurea* transcriptome and differential transcript abundance to identify putative defence related genes in challenged versus non-challenged plants. A total of 1,272,600,000 reads from 18 cDNA libraries were merged and assembled into 215,739 transcript contigs. BLASTX alignment to Nr NCBI database identified 124,386 unique annotated transcripts (57.7%) with significant hits. Functional annotation identified 83,550 out of 124,386 unique transcripts, which were mapped to 141 pathways. 39% of unigenes were assigned GO terms. Their functions cover biological processes, cellular component and molecular functions. Genes associated with response to stimuli, cellular and primary metabolic processes, catalytic and transporter functions were among those identified. Differential transcript abundance analysis using DESeq revealed significant differences among libraries depending on post-challenge times. Comparative cyto-histological studies of *P. purpurea* roots challenged with *P. cinnamomi* zoospores and controls revealed specific morphological features (exodermal strips and epi-cuticular layer), that may provide a constitutive efficient barrier against pathogen penetration. Genes involved in cutin biosynthesis and in exodermal Casparian strips formation were up-regulated. Conclusions: The de novo assembly of transcriptome using short reads for a non-model plant, *P. purpurea*, revealed many unique transcripts useful for further gene expression, biological function, genomics and functional genomics studies. The data presented suggest a combination of a constitutive resistance and an increased transcriptional response from P. purpurea when challenged with the pathogen. This knowledge opens new perspectives for the understanding of defence responses underlying pathogenic oomycete/plant interaction upon challenge with *P. cinnamomi*.

49 Metabarcoding and development of new real-time specific assays reveal *Phytophthora* species diversity in holm oak forests in eastern Spain

The evergreen holm oaks (*Quercus ilex* subsp. *ilex* and *Q. ilex* subsp. *ballota*) are the most representative tree species in the Iberian peninsula and the main tree species in oak-rangeland ecosystems (*dehesas*). Oak decline in western, central and southern parts of Spain has been associated with root rot caused by *Phytophthora cinnamomi* for decades. However, *Phytophthora* species such as *P. quercina* and *P. psychrophila* have recently been found associated with *Quercus* decline in eastern Spain where calcareous soils are predominant. Soil and root samples from two *Quercus* forests presenting decline symptoms in two different geographical areas in eastern Spain (Carrascar de la Font Roja and Vallivana) were analysed by amplicon pyrosequencing. Metabarcoding analysis showed *Phytophthora* species diversity, and revealed that an uncultured *Phytophthora taxon*, named provisionally *Phytophthora taxon ballota*, was the predominant species in both areas. In addition, a realtime PCR assay, based on the pyrosequencing results, was developed for the detection of this uncultured *Phytophthora* taxon, and also for the detection of *P. quercina*. TaqMan assays were tested on soil and root samples, and on *Phytophthora* pure cultures. The new assays showed high specificity and were consistent with metabarcoding results. A new real-time PCR protocol is proposed to evaluate the implication of different *Phytophthora* spp. in oak decline in eastern Spain.

50 **Further secondary metabolites produced by *Diplodia corticola*, a fungal pathogen involved in cork oak decline**

A new nor-pimarane diterpene and a new monosubstituted bifuranylone, named sphaeropsidin G (1) and diplobifuranylone D (2), were isolated from the organic extract of *Diplodia corticola* an oak pathogen together with the already known 4-hydroxyscytalone (3) and diorcinol (4). Sphaeropsidin G and diplobifuranylone D were characterized by using spectroscopic methods as 1,1,7-trimethyl-7-vinyl2,3,4,4a,4b,5,6,7,10,10a-decahydro-1*H*-phenanthren-9-one and 5'-(1-hydroxyethyl)-2',5'-dihydro-2*H*-[2,2']bifuranyl-5-one, respectively. The relative configurations of sphaeropsidin G and diplobifuranylone D were assigned by comparison of their spectroscopic properties with those of sphaeropsidin A and diplobifuranylones A-C and confirmed by NOESY spectra. Each compound was tested for phytotoxic, antifungal, antioomycetes and zootoxic activity. Compound 4 was active in all bioassays exhibiting remarkable phytotoxicity on *Quercus afares*, *Quercus suber*, *Quercus ilex* and *Celtis australis* leaves at 1 mg/ mL causing necrotic lesions. It completely inhibited the mycelial growth of *Athelia rolfsii*, *Lasiodiplodia mediterranea* and *Phytophthora cinnamomi*. In the *Artemia salina* assay, 1, 3 and 4 proved to be active, while 2 was found to be inactive in all bioassays.

51 **Temporal metabolic profiling of the *Quercus suber–Phytophthora cinnamomi* system by middle-infrared spectroscopy**

The oomycete *Phytophthora cinnamomi* is an aggressive plant pathogen, detrimental to many ecosystems including cork oak (*Quercus suber*) stands, and can inflict great losses in one of the greatest hotspots for biodiversity in the world. Here, we applied Fourier transform-infrared (FT-IR) spectroscopy combined with chemometrics to disclose the metabolic patterns of cork oak roots and *P. cinnamomi* mycelium during the early hours of the interaction. As early as 2h post-inoculation (hpi), cork oak roots showed altered metabolic patterns with significant variations for regions associated with carbohydrate, glycoconjugate and lipid groups when compared to mock-inoculated plants. These variations were further extended at 8hpi. Surprisingly, at 16hpi, the metabolic changes in inoculated and mock-inoculated plants were similar, and at 24hpi, the metabolic patterns of the regions mentioned above were inverted when compared to samples collected at 8hpi. Principal component analysis of the FT-IR spectra confirmed that the metabolic patterns of inoculated cork oak roots could be readily distinguished from those of mock inoculated plants at 2, 8 and 24hpi, but not at 16hpi. FT-IR spectral analysis from mycelium of *P. cinnamomi* exposed to cork oak root exudates revealed contrasting variations for regions associated with protein groups at 16 and 24h post-exposure (hpe), whereas carbohydrate and glycoconjugate groups varied mainly at 24hpe. Our results revealed early alterations in the metabolic patterns of the host plant when interacting with the biotrophic pathogen. In addition, the FT-IR technique can be successfully applied to discriminate infected cork oak plants from mock-inoculated plants, although these diferences were dynamic with time. To a lesser extent, the metabolic patterns of *P. cinnamomi* were also altered when exposed to cork oak root exudates.

52 **Quantitative RT-PCR analysis of differentially expressed genes in *Quercus suber* in response to *Phytophthora cinnamomi* infection**

cDNA-AFLP methodology was used to gain insight into gene fragments differentially present in the mRNA profiles of *Quercus suber* roots infected with zoospores of *Phytophthora cinnamomi* at different post challenge time points. Fifty-three transcript-derived fragments (TDFs) were identified and sequenced. Six candidate genes were selected based on their expression patterns and homology to genes known to play a role in defence. They encode a cinnamyl alcohol dehydrogenase2 (QsCAD2), a protein disulphide isomerase (QsPDI), a CC-NBS-LRR resistance protein (QsRPc), a thaumatin-like protein (QsTLP), a chitinase (QsCHI) and a 1,3-β-glucanase (QsGlu). Evaluation of the expression of these genes by quantitative polymerase chain reaction (qPCR) revealed that transcript levels of QsRPc, QsCHI, QsCAD2 and QsPDI increased during the first 24 h post-inoculation, while those of thaumatin-like protein decreased. No differential expression was observed for 1,3-β-glucanase (QsGlu). Four candidate reference genes, polymerase II (QsRPII), eukaryotic translation initiation factor 5A (QsEIF-5A), β-tubulin (QsTUB) and a medium subunit family protein of clathrin adaptor complexes (QsCACs) were assessed to determine the most stable internal references for qRTPCR normalization in the *Phytophthora-Q. suber* pathosystem in root tissues. Those found to be more stable, QsRPII and QsCACs, were used as internal reference in the present work. Knowledge on the *Quercus* defence mechanisms against biotic stress is scarce. This study provides an insight into the gene profiling of a few important genes of *Q. suber* in response to *P. cinnamomi* infection contributing to the knowledge of the molecular interactions involving *Quercus* and root pathogens that can be useful in the future to understand the mechanisms underlying oak resistance to soil-borne oomycetes.

53 Anatomy and development of the endodermis and phellem of *Quercus suber* L. roots

Quercus suber L. has been investigated with special attention to the stem bark and its cork formation layer, but excluding the roots. Roots are the location of infection by pathogens such as *Phytophthora cinnamomi* responsible for the tree's sudden death. It is widely accepted that suberin establishes boundaries within tissues, serves as a barrier against free water and ion passage, and works as a shield against pathogen attacks. We followed the suberization of young secondary roots of cork oak. The first suberin deposition detectable by transmission electron microscopy (TEM) and neutral red (NR) was in the endoderm Casparian strips. Casparian strips are not detected by Sudan red 7B and Fluorol yellow (FY) that specifically stain lamellae suberin. Reaction to Sudan was verified in the endodermis and later on in phellem cells that resulted from the phellogen. Under TEM, the Sudan and FY-stained cells showed clear suberin lamellae while the newer formed phellem cells displayed a distinct NR signal compared to the outermost phellem cells. We concluded that suberin chemical components are arranged differently in the cell wall according to the physiological role or maturation stage of a given tissue.

54 Physiological and proteomics analyses of Holm oak (*Quercus ilex* subsp. *ballota* [Desf.] Samp.) responses to *Phytophthora cinnamomi*

Phytophthora cinnamomi is one of the agents that trigger the decline syndrome in *Quercus* spp., this being a serious threat to Mediterranean Holm oak forest sustainability and reforestation programs. *Quercus* ilex responses to *Phytophthora cinnamomi* have been studied in one-year olds seedlings from two Andalucia provenances, assessing the physiological water status and photosynthesis-related parameters. Upon inoculation with mycelium a reduction in water content, chlorophyll fluorescence, stomatal conductance and gas exchange was observed along a 90 days post inoculation period in both provenances. The reduction was higher in the most susceptible (SSA) provenance, than in the most tolerant (PCO), being these typical plant responses to drought stress. Leaf protein profiles were analyzed in non-inoculated and inoculated seedlings from the two provenances by using a 2-DE coupled to MS proteomics strategy. Ninety seven proteins changing in abundance in response to the inoculation were successfully identified after MALDI-TOF-TOF analyses. The largest group of variable identified proteins were chloroplasts ones, and they were involved in the photosynthesis. Calvin cycle and carbohydrate metabolism. It was noted that a general tendency was a decrease in the protein abundance as a consequence of the inoculation, being it less accused in the least susceptible, the Northern provenance (PCO), than in the most susceptible, the Southern provenance (SSA). This trend is clearly manifested in photosynthesis, amino acid metabolism and stress/defence proteins. On the contrary, some proteins related to starch biosynthesis, glycolysis and stress related peroxiredoxin showed an increase upon inoculation. These changes in protein abundance were correlated to the estimated physiological parameters and have been frequently observed in plants subjected to drought stress.

55 ***In vitro* and *in vivo* quantification of elicitin expression in *Phytophthora cinnamomi***

The differential expression of four *Phytophthora cinnamomi* elicitin genes was analysed by Real Time RT-PCR. In in vitro cultures, the *α-cinnamomin* gene showed the highest level of expression, the β-cinnamomin gene (β-cin) was the most inducible, and the HAE transcripts were in low abundance. Transcription of all the elicitins was active during the active growth of the pathogen when infecting cork oak (*Quercus suber*) roots, and as host colonization progressed, the level of β-cin expression fell, while that of α-cin rose. In an antisense transgenic strain, the silencing of β-cin also negatively affected the expression of other elicitin genes in the cluster. The reduced *in planta* growth of the β-cin knock-out is related to the altered pattern of elicitin gene expression, supporting the idea that one of the functions of elicitins is related, directly or indirectly, with pathogenesis.

56 **Involvement of a cinnamyl alcohol dehydrogenase of *Quercus suber* in the defence response to infection by *Phytophthora cinnamomi***

A gene encoding a potential NADPH-dependent cinnamyl alcohol dehydrogenase (QsCAD1) (GenBank accession no: AY362455) was identified in *Quercus suber* (cork oak). Its complete cDNA sequence was obtained by RACE-PCR, starting from total RNA extracted from roots of seedlings of *Q. suber*, infected with *Phytophthora cinnamomi*, the causal agent of the decline and sudden death of *Q. sub*er and *Quercus ilex* subsp.

rotundifolia in the Iberian Peninsula. Sequence information to perform the RACE PCR was acquired from a polymorphic fragment (C9), specifically identified by cDNA-AFLP, in leaves of epicormic shoots of a cork oak tree that suffered sudden death. RT-PCR and hybridization analysis showed that the QsCAD1 gene is up-regulated in root seedlings of *Q. suber* infected with *P. cinnamomi*. QsCAD1 has a high structural homology with VR-ERE (*Vigna radiata*), an enzyme that detoxifies eutypine (produced by *Eutypa lata*, the causal agent of eutypa dieback of grapevines), to eutypinol, and with QrCAD1 (*Q. ilex* subsp. *rotundifolia*), EgCAD1 (*Eucalyptus gunnii*), MdCAD1 (*Malus* x *domestica*). Taken together, these results suggest that these enzymes, and namely QsCAD 1 belong to a new group of CAD potentially involved in deactivation of toxins produced by phytopathogens.

Artigo n.º	**Título**/Resumo
57	**Testing systemic fungicides for control of *Phytophthora* oak root disease**

Potassium phosphite (PP) formulations registered as fertilizers are now prohibited in Spain. Therefore, we evaluated the systemic fungicide fosetyl-aluminium (fos-al) in comparison with PP, against root rot caused by *Phytophthora cinnamomi* in *Quercus* woodlands. The direct effect of both systemic fungicides was evaluated *in vitro* on *P. cinnamomi* mycelial growth. Protection of cork and holm oak against infection was also evaluated in planta. Metalaxyl was included in both *in vitro* and *in planta* experiments for comparison purposes. At 100µg/mL, PP totally inhibited colony radial growth, in comparison with 75% achieved by fos-al. At doses recommended by manufacturers, with fos-al and metalaxyl applications, root symptoms remained similar to the uninfected control levels. Based on these results, fos-al is a candidate substitute product for PP in *Quercus* woodlands for control of *Phytophthora* oak root disease.

58 **Screening brassicaceous plants as biofumigants for management of *Phytophthora cinnamomi* oak disease**

Brassicaceous plants rich in glucosinolates have been used as biofumigants for the management of soilborne pathogens. Efficacy of *Brassica* plant tissue has mainly been attributed to toxic isothiocyanates released upon the hydrolysis of glucosinolates. Management of *Phytophthora cinnamomi*, the causal agent of oak root rot in rangeland ecosystems using biofumigation, is promising, but requires further validation. The biofumigation activity of 14 brassicaceous plants was evaluated under experimental conditions. All evaluated plants rich in sinigrin suppressed (100%) the mycelial growth of *P*. *cinnamomi*, while plants rich in aromatic or other aliphatic glucosinolates had little or no suppressive effect. Simulating soil amendment in field conditions, the effects on natural soil artificially infested with *P. cinnamomi* chlamydospores were examined with *Brassica juncea*, *Eruca vesicaria* and *Lepidium sativum*, three species with different glucosinolate profiles. Only *B. juncea* decreased the viability of chlamydospores significantly in comparison with untreated soil only 1 day after biofumigation, whereas *E. vesicaria* needed 8 days to reach significance and *L. sativum* had no effect at all. Despite the decreases in soil inoculum, biofumigation with *B. juncea* did not prevent the root infections in a highly susceptible host (*Lupinus luteus*). However, biofumigation with plants rich in sinigrin, such as *B. juncea*, decreased *P. cinnamomi* soil inoculum under the experimental minimum threshold for oak disease expression. Although biofumigation should be considered as an effective measure to be incorporated in integrated control of the oak disease, biofumigation by itself would not be effective enough for the substantial suppression of *P. cinnamomi* inoculum.

59 Effect of Brassica biofumigant amendments on different stages of the life cycle of *Phytophthora cinnamomi*

The oomycete plant pathogen *Phytophthora cinnamomi* causes a highly destructive root rot that affects numerous hosts. Integrated management strategies are needed to control *P*. *cinnamomi* in seminatural oak rangelands. We tested how biofumigation affects crucial stages of the pathogen's life cycle *in vitro*, in infested soils under laboratory conditions and *in planta*. Different genotypes of three potential biofumigant plant species (*Brassica carinata*, *Brassica juncea*, *Brassica napus*) were collected at different phenological stages, analysed for their glucosinolate contents, and subsequently tested. The most effective genotypes against mycelial growth and sporangial production were further tested on the viability of chlamydospores in artificially infested natural soils and in planta on *Lupinus luteus*, a host highly susceptible to *P. cinnamomi*. *Brassica carinata* and *B. juncea* genotypes inhibited mycelial growth, decreased sporangial production, and effectively inhibited the viability of chlamydospores in soil, but only *B. carinata* decreased disease symptoms in plants. Effective genotypes of *Brassica* had high levels of the glucosinolate sinigrin. Biofumigation with *Brassica* plants rich in sinigrin has potential to be a suitable tool for control of oak root disease caused by *P. cinnamomi* in Spanish oak rangeland ecosystems.

60 Anti-*Phytophthora cinnamomi* activity of *Phlomis purpurea* plant and root extracts

Phlomis purpurea (Lamiaceae), found in *Quercus suber* and *Quercus ilex* ssp. *rotundifolia* forest habitats in southern Portugal, is a nonhost for the oomycete *Phytophthora cinnamomi*, the main biotic factor involved in cork oak and holm oak decline in the Iberian Peninsula. The effect of *P. purpurea* crude ethanol root extract was evaluated *in vitro* on *P*. *cinnamomi* mycelial growth, sporangial production, zoospore release and germination as well as on chlamydospore production and viability. The protection of cork oak against infection by the pathogen was also evaluated in planta. At 10 mg ml^{-1}, in vitro inhibition of the pathogen structures was 85-100 %. In addition, *P. purpurea* plants were shown to protect *Q. suber* and *Q. ilex* from *P. cinnamomi* infection and to reduce the inoculum potential in glasshouse trials, indicating the ability to reduce root infection by the pathogen. The results suggest that *P. purpurea* has the potential to reduce disease spread and that their root extracts could provide candidate substances for control of the important pathogen, *P. cinnamomi*.

61 **Calcium mineral nutrition increases the tolerance of *Quercus ilex* to *Phytophthora* root disease affecting oak rangeland ecosystems in Spain**

Root rot caused by the soil-borne pathogen *Phytophthora cinnamomi* is leading to significant oak tree mortality in rangeland ecosystems in south western Spain. Susceptibility to *P. cinnamomi* infections of *Q. ilex* seedlings with a standard nutrition, deficient in K^+, and deficient in Ca^{2+}, was tested. Oaks deficient in K^+ showed high values in Ca^{2+} content and were tolerant to the disease. Nutritional deficiency in Ca^{2+}, however, did not lead to a higher K^+ level in plants and induced poorer root development. In addition, K^+ plant content does not appear to have any effect on pathogen tolerance. Based on these results, we conclude that satisfactory calcium nutrition may confer Holm oaks with a greater tolerance to root disease caused by *P. cinnamomi*. For this reason, limestone supplements are recommended as a measure against root rot caused by *P. cinnamomi* in rangelands in southern Spain, as a good option for control of oak root disease.

62 **Calcium fertilizers induce soil suppressiveness to *Phytophthora cinnamomi* root rot of *Quercus ilex***

Based on the observation that the root disease caused by *P. cinnamomi* on *Q. ilex* has a low incidence and severity in soils with medium high Ca^{2+} content, we studied the ability of Ca^{2+} fertilizers to induce soil suppressiveness to the pathogen. Studies on cultures of *P. cinnamomi* exposed to different Ca^{2+} fertilizers in vitro showed significant inhibition of sporangial, chlamydospore and zoospore production at millimolar concentrations while mycelial growth was mainly unaffected. Experiments performed with artificially infested soil showed that some Ca^{2+} fertilizers induce a significant decrease on chlamydospore viability. Additionally, greenhouse experiments using artificially infested soils showed a significant reduction of foliar and root symptom severities in Holm oak seedlings growing in soils amended with Ca^{2+} fertilizers. We suggest that limestone amendments in oak rangelands could enhance the suppressiveness of soils to *P. cinnamomi*, and it is likely that the inhibition of sporangial production was the main mechanism involved.

63 **Evaluating potassium phosphonate injections for the control of *Quercus ilex* decline in SW Spain: implications of low soil contamination by *Phytophthora cinnamomi* and low soil water content on the effectiveness of treatments**

The Iberian forests are suffering severe disease and mortality as a result of decline, with *Quercus ilex* the major species at risk. Trunk injections with potassium phosphonate, which have been used successfully to control *Phytophthora cinnamomi*, were tested against decline. In an area in which *P. cinnamomi* was isolated, *Q. ilex* trees showing different degrees of decline were trunk-injected. Soil properties, and measurements of soil water content (theta) and depth to soil water table were assessed at three sites with markedly different decline incidences. Over the 5 years following the initiation of the experiment, mean symptoms among spring-treated trees and autumn-treated trees, or among trees injected twice a year (spring and autumn), once a year, and non-injected, were not significantly different. No effects of the treatments on shoot growth and acorn production were observed. However, theta values under trees which recovered from decline were higher than theta values under trees which did not recover from decline. At the site with the highest incidence of decline and tree mortality, *P. cinnamomi* was rarely isolated, and the presence of gravel, soil infiltration capacities and water table depth values were significantly higher than at the other sites, water stress being more likely to contribute to decline than *P. cinnamomi*. In areas in which theta is low, the distribution of phosphonate on the tree would be limited. Since the thresholds for phytotoxicity of potassium phosphonate in *Q. ilex* trees at the site studied would be higher than the amounts used, rates of the chemical slightly less than those that cause phytotoxicity should be tested.

64 **Evaluation of biofumigant plants and organic amendments for suppressiveness of root rot of *Quercus* spp. caused by *Phytophthora cinnamomi***

Root rot caused by *Phytophthora cinnamomi* is the most serious disease of Mediterranean *Quercus* spp. in southern Spain. A strategy for disease control in natural agroecosystems is soil amendments. We examined the effects of fumigant plants (*Brassica carinata, B. juncea, Cistus albus, C. ladanifer, Diplotaxis* sp., and *Phlomys purpurea*) and fresh and composted animal manures (bovine, chicken, pig, and sheep) on mycelial growth on culture media, inoculum potential in artificially infested soil, and root infection of the susceptible host *Lupinus angustifolius*. Inoculum potential was assessed as inoculum density and colonization of *Eucalyptus* leaf pieces in soil bioassays. All treatments reduced mycelial growth of *P. cinnamomi* and complete suppression was reached by all doses of *B. carinata* and chicken manure. Most treatments also reduced inoculum potential of infested soil. Incidence of root infections in *L. angustifolius* seedlings was reduced (> 81%) by all organic amendments. The three most effective treatments under controlled conditions (*Brassica* spp., chicken manure and pig slurry) are being tested at 1.3 Kg per m^2 in two experimental fields with young trees of *Quercus rotundifolia* growing in soils highly infested by *P. cinnamomi*.

65 Treatment of oak decline using pressurized injection capsules of antifungal materials

Querus ilex and *Quercus suber* trees growing at several sites in Extremadura, Western Spain, that were showing symptoms of oak decline were injected with potassium phosphonate, quinosol or carbendazim using a low-pressure method of trunk injection composed of a pressurized capsule system. A team of four people injected between 120 and 189 trees per day, depending upon the density of the undergrowth vegetation. This labour cost represented, approximately, 15-20% of the total cost of the treatment. The potassium phosphonate-injected trees showed a significant improvement in vegetative growth within 2 years of the injection treatment, and they also showed some recovery from the decline symptoms during the third year. Only one injection treatment of an average of 3.5 capsules (corresponding to 24.5 g phosphonic acid) per tree of approximately 36 cm in diameter, was necessary to reduce the disease severity significantly. Indirectly, these results corroborate the implication of *Phytophthora cinnamomi* in oak decline within Spanish *Quercus* woodlands.

Artigo n.º	**Título**/Resumo
66	**Disentangling the climatic and biotic factors driving changes in the dynamics of *Quercus suber* populations across the species' latitudinal range**

Aim: Impacts of different global change drivers are altering the performance of plant species worldwide. However, these pressures usually differ across the species' distribution range. To properly assess the combined effect of global change at species level, we need to evaluate its consequences across their complete distribution. We focused on recent decline in Cork oak (*Quercus suber* L.) populations given its high ecological and economic relevance.

Location: We selected 10 different sites (and two populations per site) separated about one degree in latitude across the core distribution of *Q. suber*, following a transcontinental aridity gradient.

Methods: To evaluate the current trends in population dynamics across the species' distribution and the factors implied on population decline, we evaluated the effect of latitude, aridity, pathogens (*Phytophthora cinnamomi*), stand density and tree size on seed and crop size, demographic structure, dominance of recruitment bank, defoliation and mortality.

Results: We found an increase in seed weight as latitude decreased, with a homogeneous low crop size across the complete distribution. Demographic structure was determined by latitude, precipitation and pathogen abundance. We detected a trend towards reduced sapling densities towards the southern edge of the distribution, with a demographic structure dominated by old trees. The low sapling density at the southern edge translates into a loss of dominance with respect to other woody species, suggesting an alteration of community structure in the mid-term future. Tree density, precipitation and pathogen abundance determined tree mortality across the species distribution, with a higher abundance of pathogens in central-latitude populations.

Main conclusions: Our results allow the early detection of declining trends and the evaluation of the main risks for species' conservation, suggesting potential for range displacement of the species driven by the recruitment failure at the southern edge of the distribution and a likely range expansion at northern populations.

67 ***Phytophthora cinnamomi*** **as a driver of forest change: Implications for conservation and management**

Phytophthora cinnamomi is a soil-borne plant pathogen of global significance, threatening many forest tree species around the world. In contrast to other well-known tree pathogens, *P. cinnamomi* is a generalista pathogen that, in many cases, causes less immediately obvious symptoms, making *P. cinnamomi* more difficult to diagnose. This creates special challenges for those trying to assess and manage diseases caused by *P. cinnanmomi*. *P. cinnamomi* affects a wide range of tree species across the world including chestnuts, particularly American and European chestnuts, *Eucalyptus* and *Banksia* species in Australia, and oaks in Mediterranean Europe. We believe that forest professionals should incorporate an understanding of the diseases caused by *P. cinnamomi* in conservation, management, and restoration of threatened ecosystems dominated by *P.* cinnamomi-affected tree species. Here we review the impact of *P. cinnamomi* on forest ecosystems internationally and suggest three major strategies for improving forest resilience to *P. cinnamomi*: (1) Improving site quality to reduce risk of *P. cinnamomi*-related disease, (2) Genetically improving threatened species to improve resistance to *P*. *cinnamomi*, and (3) Restricting further spread (especially by nursery trade) of *P*. *cinnamomi*.

68 **Across-scale patterning of plant–soil–pathogen interactions in *Quercus suber* decline**

Forests worldwide have been recently affected by severe decline and mortality, while our understanding about forest decline across spatial scale is still limited. In this work, we study how *Quercus suber* trees adjust their physiology, in terms of water use efficiency and secondary growth, to pathogen-induced oak decline at the whole-tree, local and landscape scales. This work was carried out in Mediterranean mixed forests where their dominant key species *Q. suber* is affected by a severe decline and mortality induced by the exotic soil-borne pathogen *Phytophthora cinnamomi*. Significant differences were not observed between defoliated and healthy trees, either in terms of water use efficiency or growth at the whole-tree scale. We found that limiting conditions, such as low soil depth and high pathogen abundance, induced trees to higher water use efficiency at local and landscape scales. Overall our findings point out that *Q. suber* trees subjected to soil drought and root pathogens increase water use efficiency to some extent, while this response might not be enough for the trees to overcome the physiological stress associated with the pathogen-induced dieback. We discuss the complex way by which adult *Q. suber* trees physiologically respond to *P. cinnamomi*-induced mortality, improving our understanding of the likely consequences of chronic oak decline in the future.

69 **Plant-soil feedbacks in declining forests: implications for species coexistence**

Plant-soil feedbacks (PSFs) play a relevant role as drivers of species abundance, coexistence, and succession in plant communities. However, the potential contribution of PSFs to community dynamics in changing forest ecosystems affected by global change drivers is still largely unexplored. We measured the direction, strength and nature (biological vs. chemical) of PSFs experienced by coexisting tree species in two types of declining *Quercus* suber forests of southwestern Spain (open woodland vs. closed forest) invaded by the exotic soil pathogen *Phytophthora cinnamomi*. To test PSFs in a realistic community context, we focused not only on individual PSFs (i.e., comparing the growth of a tree species on conspecific vs. heterospecific soil) but also calculated net-pairwise PSFs by comparing performance of coexisting tree species on their own and each other's soils. We hypothesized that the decline and death of *Q. suber* would alter the direction and strength of individual and net-pairwise PSFs due to the associated changes in soil nutrients and microbial communities, with implications for recruitment dynamics and species coexistence. In support of our hypothesis, we found that the decline of *Q. suber* translated into substantial alterations of individual and net-pairwise PSFs, which shifted from mostly neutral to significantly positive or negative, depending on the forest type. In both cases however the identified PSFs benefited other species more than *Q. suber* (i.e., heterospecific positive PSF in the open woodland, conspecific negative PSF in the closed forest). Our results supported PSFs driven by changes in chemical soil properties (mainly phosphorus) and arbuscular mycorrhizal fungi, but not in pathogen abundance. Overall, our study suggests that PSFs might reinforce the loss of dominance of *Q. suber* in declining forests invaded by *P. cinnamomi* by promoting the relative performance of non-declining coexisting species. More generally, our results indicate an increase in the strength of net PSFs as natural forests become disturbed by global change drivers (e.g., invasive species), suggesting an increasingly important role of PSFs in forest community dynamics in the near future.

Artigo n.º	**Título**/Resumo
70	**Pathogen-induced tree mortality interacts with predicted climate change to alter soil respiration and nutrient availability in Mediterranean systems**

Ecosystems worldwide must simultaneously cope with several global change drivers with potential strong effects on ecosystem functioning. These drivers might interact in unexpected ways, but our still limited understanding of these interactive effects precludes us from predicting the impact of global change on ecosystem functioning. In this study we assessed the direct effects of pathogen-induced tree mortality and predicted warming and drought on C, N and P in Mediterranean forest soils affected by the decline of their dominant tree species (i.e. *Quercus suber*) due to the invasive pathogen *Phytophthora cinnamomi*. We also explored the potential indirect effects due to species replacement after *Q. suber* mortality. To achieve our goal, we conducted a soil incubation experiment using soils collected under *Q. suber* trees with different health status (i.e. healthy, defoliated and dead trees) and from coexistent shrubs (i.e. pioneer and late successional shrubs). These soils were incubated under controlled temperatures and soil moistures, mimicking various climate change scenarios predicted for 2050 and 2100 in the Mediterranean Basin. Our results showed that *P. cinnamomi*-induced mortality and future warming and drought may interact to simultaneously alter biogeochemical cycles in *Q*. *suber* forest soils. Resistance of studied variables to changes in temperature and moisture tended to be lower for dead trees than for healthy and defoliated trees. Moreover, we found that soil respiration and nutrient availability might be affected indirectly by *P*. *cinnamomi*-induced mortality due to species replacement. Overall, our results support a high potential of invasive pathogen species for modifying the response of soil functioning to climatic stressors.

71	**Fine scale determinants of soil litter fauna on a Mediterranean mixed oak forest invaded by the exotic soil-borne pathogen *Phytophthora cinnamomi***

There is growing recognition of the importance of soil fauna for modulating nutrient cycling processes such as litter decomposition. However, little is known about the drivers promoting changes in soil fauna abundance on a local scale. We explored this gap of knowledge in a mixed oak forest of Southern Spain, which is under decline due to the invasion of the exotic soil-borne pathogen *Phytophthora cinnamomi*. Mesoinvertebrate abundance found in soil litter was estimated at the suborder level. We then explored their statistical correlations with respect to light availability, tree and litter characteristics, and *P*. cinnamomi abundance. *Oribatida* and *Entomobryomporpha* were the most abundant groups of *Acari* and *Collembola*, respectively. According to their trophic level, predator and detritivore abundances were positively correlated while detritivores were, in turn, positively correlated with pathogen abundance and negatively influenced by light availability and tree defoliation. These overall trends differed between groups. Among detritivores, *Diplopoda* preferred highly decomposed litter while *Oribatida* and *Psocoptera* preferred darker environments and *Poduromorpha* were selected for environments with lower tree defoliation. Our results show the predominant role of light availability in influencing litter fauna abundances at local scales and suggest that the invasive soil-borne pathogen *P. cinnamomi* is integrated in these complex relationships.

72 ***Quercus suber* dieback alters soil respiration and nutrient availability in Mediterranean forests**

1. An increase in tree mortality rates has been recently detected in forests world-wide. However, few works have focused on the potential consequences of forest dieback for ecosystem functioning. **2.** Here we assessed the effect of *Quercus suber* dieback on carbon, nitrogen and phosphorus cycles in two types of Mediterranean forests (woodlands and closed forests) affected by the aggressive pathogen *Phytophthora cinnamomi*. We used a spatially explicit neighbourhood approach to analyse the direct effects of *Q. suber* dieback on soil variables, comparing the impact of *Q. suber* trees with different health status, as well as its potential long-term indirect effects, comparing the impact of non-declining coexistent species. **3.** *Quercus suber* dieback translated into lower soil respiration rates and phosphorus availability, whereas its effects on nitrogen varied depending on forest type. Coexistent species differed strongly from *Q. suber* in their effects on nutrient availability, but not on soil respiration rates. Our models showed low interannual but high intra annual variation in the ecosystem impacts of tree dieback. **4.** Synthesis. Our results support that tree dieback might have important short- and long-term impacts on ecosystem processes in Mediterranean forests. With this work, we provide valuable insights to fill the existent gap in knowledge on the ecosystem-level impacts of forest dieback in general and *P. cinnamomi*-driven mortality in particular. Because the activity and range of this pathogen is predicted to increase due to climate warming, these impacts could also increase in the near future altering ecosystem functioning world-wide.

73 **Combined effects of soil properties and *Phytophthora cinnamomi* infections on *Quercus ilex* decline**

The importance of soil properties as determinants of tree vitality and *Phytophthora cinnamomi* root infections was analysed. The study comprised 96 declining stands in western Spain, where declining and non declining holm oak (*Quercus ilex* L.) trees were sampled. Soil properties (soil depth, Ah horizon thickness, texture, pH, redox potential, soil bulk density and N-NH_4^+ and N-NO_3^- concentrations) and *P. cinnamomi* infections were assessed. Tree mortality rates increased with low soil bulk densities, which were also associated with more *P. cinnamomi*-infected trees. Occurrence of infected trees was higher in fine textured soils and in thick Ah horizons. Fine textured soils favoured trees, but with the presence of *P. cinnamomi* their health status deteriorated. Soil under declining trees had higher N-NO_3^-/N-NH_4^+ ratio values than under non-declining trees. Additional soil properties changes associated to grazing were not related to decline and *P. cinnamomi* infections. The implications of *P. cinnamomi* in holm oak decline and the influence of soil properties as contributors to pathogen activity were demonstrated. Fine soil textures and thick Ah horizons, usually favourable for vigour and vitality of trees growing in the Mediterranean climate, were shown to be disadvantageous soil properties if *P. cinnamomi* was present. Fine soil textures and thick Ah horizons are frequently related with higher levels of soil moisture, which increase the inoculum of the pathogen and favours root infection. Grazing does not seem to be directly linked to *Q. ilex* health status or *P. cinnamomi* root rot.

74 **Spatial patterns of soil pathogens in declining Mediterranean forests: implications for tree species regeneration**

Soil-borne pathogens are a key component of the belowground community because of the significance of their ecological and socioeconomic impacts. However, very little is known about the complexity of their distribution patterns in natural systems. Here, we explored the patterns, causes and ecological consequences of spatial variability in pathogen abundance in Mediterranean forests affected by oak decline. We used spatially explicit neighborhood models to predict the abundance of soil-borne pathogen species (*Phytophthora cinnamomi*, *Pythium spiculum* and *Pythium* spp.) as a function of local abiotic conditions (soil texture) and the characteristics of the tree and shrub neighborhoods (species composition, size and health status). The implications of pathogen abundance for tree seedling performance were explored by conducting a sowing experiment in the same locations in which pathogen abundance was quantified. Pathogen abundance in the forest soil was not randomly distributed, but exhibited spatially predictable patterns influenced by both abiotic and, particularly, biotic factors (tree and shrub species). Pathogen abundance reduced seedling emergence and survival, but not in all sites or tree species. Our findings suggest that heterogeneous spatial patterns of pathogen abundance at fine spatial scale can be important for the dynamics and restoration of declining Mediterranean forests.

Artigo n.º	Título/Resumo
75	**Susceptibility of common herbaceous crops to *Phytophthora cinnamomi* and its influence on *Quercus* root rot in rangelands** The susceptibility to *Phytophthora cinnamomi* of four crops (wheat, oat, vetch, and yellow lupin) commonly planted in rangeland ecosystems in southern Spain was evaluated. By means of *in vitro* infection experiments, the presence of the pathogen into the roots of yellow lupin (symptomatic) and vetch (asymptomatic) was observed, but never into wheat and oat roots (asymptomatic). It was also demonstrated that yellow lupin stimulated the production of zoospores of *P. cinnamomi*. Vetch, wheat and oat did not stimulate zoospore production. Under controlled conditions, only yellow lupin induced an increase in the number of viable chlamydospores in the soil. We concluded that the culture of wheat, oat, and vetch in rangelands did not influence the epidemiology of the *Quercus* root disease, even when asymptomatically-infected vetch is grown, and these crops can constitute an alternative to the culture of yellow lupin in rangeland ecosystems affected by *Quercus* root rot.
76	**The role of yellow lupin (*Lupinus luteus*) in the decline affecting oak agroforestry ecosystems** *Phytophthora cinnamomi* is a soilborne pathogen causing root rot in Mediterranean *Quercus* species growing in "dehesa" rangeland ecosystems. Recently, it has been reported causing wilting and death of *Lupinus luteus* (yellow lupin), a spontaneous plant in southern Spain rangelands, but also frequently sowed for livestock grazing. In soils artificially infested with *P. cinnamomi* chlamydospores and planted with different cultivars of yellow lupin, a significant increase in the density of propagules was detected in comparison with the initial levels of inoculum and with the infested but not planted soil (control). In oak rangelands in which yellow lupine was planted, isolation and counting of colonies of *P. cinnamomi* from soil samples have shown the ability of this plant to maintain or even increase the inoculum density and thus facilitate the infection of trees. Results suggested that cultivation of yellow lupin in oak-rangeland ecosystems should be avoided whether oak trees are affected by root disease caused by *P. cinnamomi* or not. This leguminous plant can act as an inoculum reservoir or even

enhance inoculum soil levels available for oak root infections, exacerbating the oak decline severity in the region.

77 **_Lupinus luteus_, a new host of _Phytophthora cinnamomi_ in Spanish oak-rangeland ecosystems**

Phytophthora cinnamomi is an aggressive pathogen on *Lupinus luteus* (yellow lupin), causing root rot, wilting and death of this crop, common in oak-rangeland ecosystems ('dehesas') in south western Spain. The oomycete, the main cause of *Quercus* decline in the region, was isolated from roots of wilted lupins in the field. Artificial inoculations on four cultivars of *L. luteus* reproduced the symptoms of the disease, both in pre- and post-emergence stages, recovering the pathogen from necrotic roots. These results suggest the potential of yellow lupin as inoculum reservoir for the infection of *Quercus* roots. This is the first report of *P. cinnamomi* as root pathogen of *L. luteus*.

78 **Structure, anti-_Phytophthora_ and anti-tumor activities of a nortriterpenoid from the rhizome of _Phlomis purpurea_ (Lamiaceae)**

To investigate bioactive compounds potentially involved in the biotic interactions exhibited by *Phlomis purpurea* (Lamiaceae) in rhizospheres infested with *Phytophthora cinnamomi*, the plant rhizome was chemically analysed. The nortriterpenoid (17S)-2α,3α,11α,23,24-pentahydroxy-19(18→17)-*abeo*-28-norolean-12-en-18-one, was isolated and its structure was elucidated by comprehensive spectroscopic analysis, chiefly using 2D NMR experiments, and X-ray analysis. It was shown to be exuded by roots and to exhibit anti-*Phytophthora* and antitumor activities.

79 **Suppression of _Phytophthora cinnamomi_ in potting mixes amended with uncomposted and composted animal manures**

We examined the effects of fresh and composted animal manures on the development of root rot, dieback, and plant death caused by *Phytophthora cinnamomi*. Fresh chicken manure, or chicken manure composted for 5 weeks before incorporation into the potting mix (25%, vol/vol), significantly reduced pathogen survival and the development of symptoms on *Lupinus albus* seedlings. Chicken manure composted for 2 weeks was less suppressive. Cow, sheep, and horse manure, whether fresh or composted, did not consistently suppress populations of *P. cinnamomi* or disease symptoms at the rates used (25%, vol/vol). All composts increased organic matter content, total biological activity, and populations of actinomycetes, fluorescent pseudomonads, and fungi. Only chicken manure stimulated endospore-forming bacteria, a factor that was strongly associated with seedling survival. Fallowing the potting mix for an additional 8 weeks after the first harvest increased the survival of lupin seedlings in a second bioassay, with survival rates in chicken manure compost-amended potting mix exceeding 90%. These data suggest that the ability of composted manure to stimulate sustained biological activity, in particular the activity of endospore-forming bacteria, is the key factor in reducing disease symptoms caused by *P. cinnamomi*. Supporting these results, the survival of rooted cuttings of *Thryptomene calycina* was significantly higher in sand-peat potting mix following amendment with commercially available chicken manure (15% vol/vol). However, this protection was reduced if the potting mix was steam pasteurized before amendment, indicating that suppression was due to endogenous as well as introduced microbes. Chicken manure compost incorporated at 5% (vol/vol) or more was strongly phytotoxic to young *Banksia spinulosa* plants and is not suitable as an amendment for phosphorus-sensitive plants.

ANEXO III

Nomes científicos e comuns das espécies vegetais referidas no texto.

Nome científico	**Nome comum**
Avena sativa L.	aveia
Calluna vulgaris (L.) Hull	torga-ordinária
Cistus ladanifer L.	esteva
Cistus populifolius L.	estevão
Cistus salviifolius L.	sargaço
Daphne gnidium L.	trovisco
Erica arborea L.	urze-branca
Erica lusitanica Rudolphi	urze-branca
Eucalyptus dunnii Maiden	eucalipto-cidra
Eucalyptus fraxinoides Deane & Maiden	
Eucalyptus marginata Sm.	
Genista triacanthos Brot.	tojo-molar
Helichrysum stoechas (L.) Moench	perpétua-das-areias
Lavandula stoechas L.	rosmaninho
Lupinus albus L.	tremoço-branco
Lupinus luteus L.	tremocilha
Myrtus communis L.	murta
Phlomis purpurea L.	marioila
Pinus taeda L.	
Quercus alba L.	carvalho-branco
Quercus canariensis Willd.	carvalho-de-Monchique
Quercus cerris L.	carvalho-da-Turquia
Quercus elliptica Née	
Quercus faginea Lam.	carvalho-cerquinho
Quercus frainetto Ten.	carvalho-da-Hungria
Quercus glaucescens Bonpl.	
Quercus glaucoides M.Martens & Galeotti	
Quercus hartwissiana Steven	
Quercus rotundifolia Lam.	azinheira
Quercus ithaburensis Decne.	carvalho-do-Monte-Tabor
Quercus macranthera Fisch. & C.A.Mey. ex Hohen.	carvalho-caucasiano
Quercus magnoliifolia Née	
Quercus montana Willd.	

Nome científico	Nome comum
Quercus palustris Münchh.	carvalho-dos-pântanos
Quercus peduncularis Née	
Quercus petraea (Matt.) Liebl.	carvalho-alvar
Quercus pyrenaica Willd.	carvalho-negral
Quercus robur L.	carvalho-alvarinho, carvalho-roble
Quercus rubra L.	carvalho-americano
Quercus salicifolia Née	
Quercus suber L.	sobreiro
Quercus virgiliana (Ten.) Ten.	
Quercus vulcanica Boiss. & Heldr. ex Kotschy	
Triticum aestivum L.	trigo
Vicia sativa L.	ervilhaca