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Distinctive population structure of *Colletotrichum* species associated with olive anthracnose in the Algarve region of Portugal reflects a host-pathogen diversity hot spot

Running title:
Olive anthracnose host-pathogen diversity hot spot

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Abstract
Anthracnose (*Colletotrichum* spp.) is an important disease of olive fruits. Diversity and biogeographic relationships of the olive anthracnose pathogens in Algarve (Portugal) were investigated along with disease levels during 2004-2007. Diverse *C. acutatum* and *C. gloeosporioides* populations were identified based on rRNA-ITS and partial β-tubulin 2 gene sequences of 95 isolates. Spatial and temporal variation in the occurrence of the eight genetic entities of the pathogens was linked to olive biogeography. Disease occurrence patterns suggest that *C. acutatum* populations are more stable pathogens, while *C. gloeosporioides* populations seem more influenced by favourable conditions. Three unique *C. acutatum* populations were identified but, none of the eight populations were dominant, with the most frequent type representing only 27%. Thus the population structure of olive anthracnose pathogens in Algarve is distinct from other parts of Portugal and other world locations, where only one or two genetic entities are dominant. This pattern and level of genetic diversity in a restricted area, where oleaster (wild olive tree), ancient landraces and modern cultivars of olive occur in close proximity, suggests Algarve as a centre of diversity of the anthracnose pathogens and corroborates recent work suggesting western Mediterranean as an important centre of olive diversity and domestication.

Keywords: *Colletotrichum acutatum*; *Colletotrichum gloeosporioides*; olive anthracnose; population structure; biogeography; olive domestication.

Introduction

Olive (*Olea europaea* ssp. *europaea* var. *europaea*) anthracnose is a very common and severe disease in Portugal, mainly caused by *Colletotrichum acutatum* (97%) and sporadically (3%) by *C. gloeosporioides* (Talhinhas *et al*., 2005). Olive anthracnose has also been reported in other central and western Mediterranean countries, Australia and South Africa. Symptoms typically occur on mature fruits (during autumn), as dark necroses with abundant orange conidial masses, leading to premature fruit drop or mummification, damaged fruits and poor oil quality (high acidity). Under favourable conditions symptoms can also occur on branches and leaves, leading to necroses, severe defoliation and death of branches. Vegetative organs play an important role in pathogen survival and multiplication and dissemination of inoculum to fruits (Talhinhas *et al.*, 2006).

The most common Portuguese olive cultivar, 'Galega', is very susceptible to anthracnose. However, the disease can also seriously affect less susceptible varieties under favourable environmental conditions and high inoculum pressure, even when the

recommended protection measures are followed. This was clearly evident following mild and wet weather conditions in autumn 2006, when important losses were reported from cultivars such as 'Arbequina' and 'Picual', previously regarded as moderately resistant, and widely cultivated throughout Iberia. This poses a threat to oliviculture even in regions where the disease incidence is currently low, suggesting that the use of a resistant variety and the application of appropriate control measures may not be enough to guarantee protection against the disease. The existence of a widespread inoculum reservoir on neglected olive orchards and on oleaster (wild olive tree, *Olea europaea* ssp. *europaea* var. *sylvestris*) in Portugal is considered the main reason for the disease being so common, and for frequently causing severe losses (Talhinhas *et al*., 2006). Previous work showed that olive anthracnose populations in Portugal are vastly dominated (85%) by the genetic group A2 of *C. acutatum*, while other groups and *C. gloeosporioides* occurred at low frequency (Talhinhas *et al*., 2005). Similarly, group A2 of *C. acutatum* was dominant in Spain, while *C. gloeosporioides* was in minority (Martín *et al*., 2002; Talhinhas *et al*., 2005). Olive anthracnose pathogens were assigned to *C. acutatum* group A9 in Australia (Whitelaw-Weckert *et al.*, 2007). In South Africa, the disease seems to be caused only by isolates belonging to *C. acutatum* group A5 (Gorter, 1956). Interestingly, in Sicily (Italy), *C. gloeosporioides* was reported as the sole olive anthracnose pathogen (Scarito *et al.*, 2003), while in Apulia (Italy) the disease is caused by *C. gloeosporioides* as well as isolates most likely belonging to *C. acutatum* group A4, based on the clustering of isolate CBS193.32 in two different studies (Agosteo *et al*., 2002; Talhinhas *et al*., 2005). Such A4 isolates were also found in other Italian regions (Calabria and Sardinia) and in Montenegro (Agosteo *et al*., 2002; Talhinhas *et al*., 2005). Preliminary data suggested a diverse population composition at Algarve compared to other parts of the world. This study investigated the population structure of the olive anthracnose pathogens in the Algarve and biogeographic relationships, including host diversity.

Materials and Methods

Sampling locations

Surveys were conducted in the Algarve over four years (2004-2007) at fruit maturity, based on olive cultivation frequency (Fig. 1a) and distribution of oleaster (Pedro, 1991). In total, 133 different sites were surveyed including repetitive visits to some sites over different years.

Assessment of disease symptoms, incidence and severity

In each collection site, the presence/absence of anthracnose symptoms on fruits was recorded. Further, asymptomatic presence of inoculum was assessed by incubating symptomless fruits in wet chamber (100% relative humidity, 22ºC) for 1-3 weeks to induce symptoms. Disease incidence was calculated as the proportion of orchards where symptoms were recorded, compared to the total number of orchards surveyed. Disease severity was scored as the proportion (%) of diseased fruits compared to total number of fruits sampled.

Agronomic, biogeographic and botanical data

For each site, the following data were recorded: exact location enabling retrieval of mapped data such as altitude and soil type; local and general topography; phytosociology - trees isolated, scarce or frequent, either in urban areas, among other crops, among other non-cultivated plants or in dominant populations (olive orchard/oleaster bushland); tree botanical/agronomical status: oleaster (*Olea europaea* ssp. *europaea* var. *sylvestris*) or oleaster-like (Belaj *et al*., 2007), ancient

cultivars/landraces in long-time abandoned inland farms, modern cultivars ('Galega', 'Maçanilha' and others). Administrative limits, hipsometry, soil type and phytogeography data were obtained from Instituto do Ambiente (Portugal) and assembled using ArcView 3.1 (ESRI).

Isolation of the pathogens

The pathogen was isolated from spore masses on fruits with anthracnose symptoms, onto Petri dishes containing Potato Dextrose Agar (PDA, Difco) amended with a bacterial growth inhibitor (KCNS 50 mM). Conformity with *Colletotrichum* spp. was verified according to colony characteristics. Single-spore cultures were obtained and stored, comprising a collection of 95 *Colletotrichum* spp. isolates. In some cases, more than one isolate was obtained from a single collection site, single tree or even from a single fruit. Koch's postulates were verified through assays on mature fruits and pathogen re-isolation.

Genetic diversity analysis based on rRNA-ITS region and partial β-tubulin 2 gene nucleotide sequence

For each isolate, species and infra-specific diversity (genetic group) were identified by molecular analyses. DNA was extracted using a rapid freeze-boil protocol (Talhinhas *et al.*, 2008). For each isolate, the species (*Colletotrichum acutatum* or *C. gloeosporioides*) was identified by PCR amplification of part of the β–tubulin 2 (*tub2*) gene using species-specific primers (Talhinhas *et al*., 2005). Infra-specific diversity among *C. acutatum* isolates was assessed by PCR-RFLP of *tub2*, assigning isolates to various groups (Talhinhas *et al*., 2005). For detailed intra-group diversity analysis, nucleotide sequences were obtained for the rRNA-ITS region and *tub2* (Talhinhas *et al*., 2002).

Results and Discussion

Population composition of the olive anthracnose pathogens

Diagnostic PCR based on a 550 bp *tub2* gene fragment was performed with each of the 95 isolates collected and 79 belonged to *C. acutatum* (83%) and 16 to *C. gloeosporioides* (17%). Among *C. acutatum* isolates, RFLP analysis of *tub2* revealed that 32 clustered in A2 (34%), 26 in A4 (27%), 19 in A5 (20%) and two in A3 (2%), based on previously described genetic groups (Sreenivasaprasad & Talhinhas, 2005; Talhinhas *et al.*, 2005). This pattern contrasts markedly to the rest of Portugal and the emerging pattern from other parts of the world, where single genetic entities are vastly dominant (Talhinhas *et al*., 2005). Therefore, the population composition of the olive anthracnose pathogens in Algarve is unique by the occurrence of *C. acutatum* groups A2, A4 and A5 and *C. gloeosporioides* at relatively comparable frequencies (Fig. 1a). Another distinct feature is the detection of *C. acutatum* group A5 isolates, distinguishable by their salmon/purple-coloured colonies. Pathogens clustering in group A5 were never reported from field crops in the northern hemisphere (Sreenivasaprasad & Talhinhas, 2005), but are common in the southern hemisphere including the *C. acutatum* type specimen (IMI117617 and ATCC56816) from papaya (Simmonds, 1965), the olive anthracnose pathogen in South Africa (Gorter, 1956) and occurring asymptomatically in association with the coffee berry disease in Angola.
rRNA-ITS sequences revealed further diversity within some of the genetic entities (Table 1). Three sub-groups (A2-1, A2-2 and A2-3), differing in 1-2 nucleotides, were found within *C. acutatum* group A2. Sub-group A2-2 on olives in general and sub-group A2-3 on any host globally have not yet been reported. No differences were found within the remaining *C. acutatum* groups. *C. gloeosporioides* isolates divided into CG-1 and CG-2, differing in one nucleotide. Analysis of *tub2* nucleotide sequence revealed no

differences between A2-1 and A2-2, but they differed in 3 bp from A2-3; no differences were found between CG-1 and CG-2. Sequences representing each genetic entity were deposited in EMBL (Table 1).

Anthracnose incidence and pathogen dynamics

Over the four year survey period (2004-2007), anthracnose incidence was 65-70% in Algarve, with another 10-15% samples showing symptoms upon incubation in wet chamber. Therefore, the pathogens were absent from only 15-25% of orchards. However, following prolonged rain, high humidity and mild temperature periods in autumn 2006, symptoms were found at all sites (100% incidence). Average disease severity was 22% in 2004 and 2005, 85% in 2006 and 36% in 2007. Although meteorological conditions during autumn 2007 were not more favourable than 2004 and 2005, the higher disease severity recorded is likely due to inoculum abundance resulting from the 2006 outbreak. Anthracnose incidence and severity was much lower on oleasters than on olives. The pathogen was not present on over 60% of oleasters and the average disease severity was only 2%, excluding 2006 when it was 60% under very favourable environmental conditions.

Average disease severity was 38-47% for the main *C. acutatum* groups and 30% for *C. gloeosporioides*. However, during 2006-07, when the overall anthracnose incidence was higher compared to 2004-05, the difference in severity was much more accentuated for *C. gloeosporioides* than for *C. acutatum* (Fig. 2b). This suggests that *C. gloeosporioides* acts as an opportunistic pathogen, responding to favourable environmental conditions and high inoculum pressure, while the diverse *C. acutatum* groups are more stable pathogens. In fact, nearly 25% of *C. gloeosporioides* isolates were obtained from symptomless fruits. This agrees with observations from Sicily (Italy) reporting *C. gloeosporioides* as a weak opportunistic olive pathogen (Agosteo *et al.*, 2002).

Frequencies of the different olive anthracnose pathogen populations varied through the years (Fig. 2a). Occurrence of *C. gloeosporioides* varied between minimum 8% (2006) and maximum 24% (2005). Groups A2 and A4 of *C. acutatum* were 20-43%, alternating as the most frequent populations, except in 2007, when group A5 was the most frequent. In fact, A5 populations showed a steady increase from 9% in 2004 to 34% in 2007. Within the Algarve, A3 isolates were identified for the first time at Faro and Silves. At Faro, the pathogen was isolated from fruits exhibiting 60% disease severity, with typical anthracnose symptoms. At Silves, the fungus was isolated together with *C. gloeosporioides*, making it difficult to associate to the symptoms or disease severity value observed. In the past, *C. acutatum* group A3 was only detected at a single location (Torres Vedras) from asymptomatic infections (Talhinhas *et al*., 2005). Previous surveys at Silves since 2001 identified only group A2, suggesting group A3 as an emerging population in Algarve. Another interesting feature is the presence of different genetic entities at single locations, sometimes on the same tree or even on the same fruit (Fig. 1a). These observations clearly show the strong influence of agroecological conditions on the diversity and dynamics of the olive anthracnose pathogens.

Biogeography of olive cultivation and anthracnose pathogens

The Algarve is crossed by a clear border line between two biogeographic provinces, the Gaditano-onubo-algarvian to the south and the Luso-extremaduran to the north (Fig. 1d; Costa *et al*., 1998). South of this line is the Algarvic superdistrict dominated by calcareous-derived soils (Fig. 1c), rich in paleomediterranic endemic vegetation, where the phytosociological community *Oleo-Quercetum suberis* is frequent. This area represents a glaciation refuge and a confluence point of floristic migratory routes. This contrasts markedly with the area to the North, mostly the Serrano-monchiquense superdistrict, which is climatically more continental, dominated by steep hills (Fig. 1b)

of thin schist-derived soils (Fig. 1c). Phytosociological associations such as *Myrto-Quercetum suberis*, *Sanguisorbo-Quercetum suberis*, *Querco lusitanicae-Stauracanthetum boivinii* and *Erico australis-Cistetum populifolii* are common here (Costa *et al*., 1998). Olive cultivation represents 1.8% (ca. 8800 ha) of the total Algarve area, contrasting with a national value of 3.4% (INE, 2001; Fig. 1a).Most (67%) of the olive cultivation occurs in the calcareous-derived soils (Fig. 1c) of the Algarvic superdistrict, locally known as 'Barrocal' (Fig. 1d). This is an undulating region (Fig. 1b), with important fruit tree cultivation, particularly almond, carob and citrus, with typical Mediterranean conditions. Most common cultivars in Algarve are 'Galega' and 'Maçanilha' (Pedro, 1991) but, to our knowledge, no detailed up-to-date information on the relative importance of cultivars has been published.

Population structure of the olive anthracnose pathogens revealed varied biogeographic relationships, although geographic variation in disease incidence and severity was limited. *C. gloeosporioides* and *C. acutatum* group A2 occurred throughout the Algarve, while *C. acutatum* groups A4 and A5 were more frequent in central Algarve (Fig. 1a). Interestingly, all genetic entities identified were found in central Algarve. However, only *C. acutatum* groups A2 and A5 were isolated from oleaster plants, regardless of location (Fig. 2c). The frequency of these groups is higher on oleaster compared to landraces and modern cultivars. On olive trees representing ancient cultivars/landraces, four main genetic entities (*C. gloeosporioides* and *C. acutatum* groups A2, A4 and A5) occurred at frequencies of 17-32%. On modern cultivars, *C. acutatum* group A5 occurred at a lower frequency (8%) and the other main genetic entities ranged from 21% (*C. gloeosporioides*) to 38% (*C. acutatum* group A2). This suggests differences in the adaptive potential of various populations to different host genetic backgrounds. Within *C. gloeosporioides*, isolates belonging to groups CG-1 (54%) and CG-2 (46%) were evenly distributed across the region, over different years and hosts. However, while all CG-1 isolates were associated with low disease severity, some CG-2 isolates were obtained from plants with up to 100% disease severity. ITS sequences of both CG-1 and CG-2 are similar to those of pathogens causing anthracnose on several hosts (e.g., avocado, rubber, strawberry and Stylosanthes) throughout the world (Sreenivasaprasad & Talhinhas, 2005). Within *C. acutatum*, majority of group A2 isolates belonged to sub-group A2-1 (75%), while A2-2 (17%) and A2-3 (8%) were less common. However, all the A2 sub-groups occurred across the region, over different years and types of hosts and were not associated with any differences in disease severity. Interestingly, A2-1 ITS sequence is identical to that of isolates causing anthracnose on several hosts, e.g. strawberry. A2-2 isolates are rare and only identical to a single isolate from photinia (accession number AJ749676). Moreover, A2-3 isolates are unique in a collection of over 150 *C. acutatum* group A2 isolates (Sreenivasaprasad & Talhinhas, 2005). Isolates belonging to *C. acutatum* group A4 cluster together with olive pathogens from Italy (CBS193.32 –accession AJ749688) and Montenegro (AJ749689 and AJ749690). ITS sequence of the *C. acutatum* group A3 isolates from olive did not differ from the vast majority of group A3 isolates, comprising pathogens of apple, blueberry, grapevine, peach and strawberry (Sreenivasaprasad & Talhinhas, 2005). The low frequency of group A3 on olive suggests that it may have originated from adjoining crops. These isolates were pathogenic to olives in artificial inoculation experiments (Talhinhas *et al.*, unpublished). Olive anthracnose pathogens clustering in *C. acutatum* group A5 did not differ in their ITS sequence from isolates from several other hosts.

Host gene pool and pathogen diversity

Olea europaea ssp. *europaea* gene pool in the Algarve, and particularly in the 'Barrocal', is comprised of modern cultivars grown in orchards and among other crops, ancient cultivars/landraces in neglected or long-time abandoned inland farms and oleaster or oleaster-like plants (Pedro, 1991). Although *Olea europaea* ssp. *europaea* is believed to have its origin in eastern Mediterranean, recent research suggests that

current distribution of oleaster would have arisen from glacial refuges both in east and west Mediterranean. One of those seven estimated refuges is in Iberia. Moreover, the genetic diversity in the west is expected to be higher compared to the east, rejecting the hypothesis that western oleasters would be feral forms of eastern oleaster-like populations (Breton *et al*., 2006a). This questions the geographic origin of *Olea europaea* ssp. *europaea* (Breton *et al*., 2006b), while archaeological data also question its place of domestication (Breton *et al*., 2006b), suggesting that it may have also occurred in western Iberia (Figueiral & Terral, 2002). Interestingly, the above-mentioned archaeological data arise from an excavation in Estremadura, one of the two main Portuguese calcareous massifs, where wild and cultivated olive trees are currently found. The other main massif is in the Algarve. Samples from this region have been rare or absent from *Olea europaea* phylogenetic investigations, although the importance of west Mediterranean for olive diversity and domestication has been highlighted (e.g., Contento *et al*., 2002; Breton *et al*., 2006a; Besnard *et al.*, 2002, 2007).

Clearly, diversity of the olive anthracnose pathogens is high in the Algarve comprising eight different populations, but no single population was dominant (Table 1). *C. acutatum* group A4 was the most frequent (27%), followed by sub-group A2-1 (25%), group A5 (20%), *C. gloeosporioides* groups CG-1 (9%) and CG-2 (8%), *C. acutatum* sub-groups A2-2 (6%) and A2-3 (3%) and group A3 (2%). At Trás-os-Montes in Portugal, A2-1 and A4-1 of *C. acutatum* were dominant representing ca. 40% each of the pathogen population. In the rest of Portugal, A2-1 represented 94% of the *Colletotrichum* spp. populations (Talhinhas *et al*., 2005). Reports so far suggest little or no intra-regional olive anthracnose population heterogeneity throughout the world (Gorter, 1956; Agosteo *et al*., 2002; Scarito *et al.*, 2003; Talhinhas *et al*., 2005; Whitelaw-Weckert *et al.*, 2007). For example, *C. acutatum* sub-group A4-1 in Calabria and Sardinia (Italy) and Montenegro; *C. gloeosporioides* in Sicily (Italy); both *C. acutatum* sub-group A4-1 (79%) and *C. gloeosporioides* (21%) in Apulia (Italy); group A5 in South Africa and group A9 in Australia have been identified as the cause of olive anthracnose. Within *C. acutatum,* groups A2 and A4 are considered as the two farthest lineages (Sreenivasaprasad & Talhinhas, 2005). Groups A3, A5 and A9 are closer to A2, while A6 is closer to A4 (Table 1). Interestingly, A2-1, A2-2, A2-3, A3 and A5, all related to the A2 lineage, along with sub-group A4-1 and *C. gloeosporioides* were identified in Algarve. This suggests wider diversity in the Algarve region of groups related to the *C. acutatum* A2 lineage compared to the A4 lineage, which is more diverse at Trás-os-Montes, where anthracnose incidence has only recently become frequent.

Eight different populations of the olive anthracnose pathogens *C. acutatum* and *C. gloeosporioides* displaying distinct structure and varied biogeographic association patterns have been identified in the Algarve. The high level of pathogen genetic variability coinciding with increased host gene pool suggests the Algarve as a centre of pathogen diversity and corroborates recent work suggesting western Mediterranean as an important centre of olive diversity and domestication. It is well recognised that diversity of pests and pathogens tends to be higher in the centres of diversity of their hosts. Phylogenetic knowledge of olives could further improve our understanding of the evolution and adaptation of anthracnose pathogens, as was reported for another major pest, the olive fly (Nardi *et al*., 2005).

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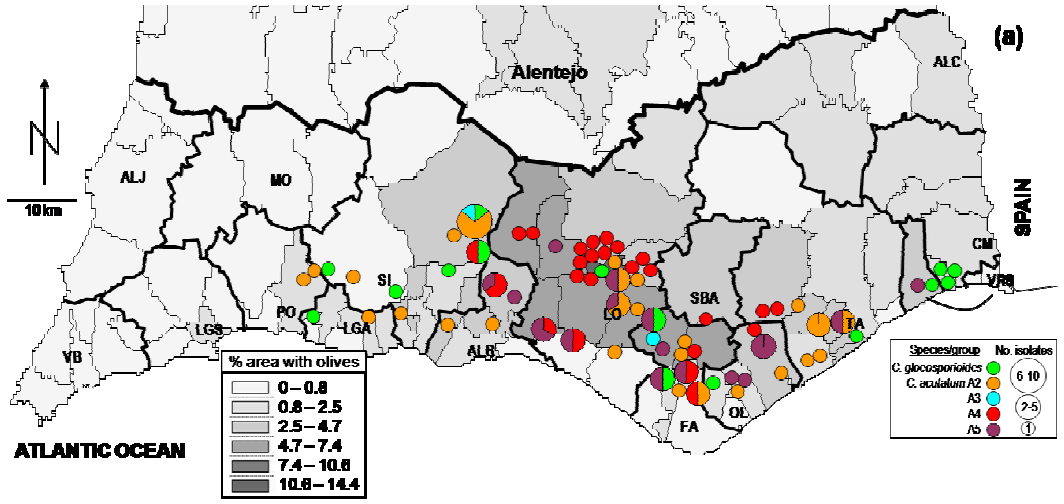
Table 1. rRNA-ITS nucleotide sequence distance coefficients based on Kimura 2-P model and database references for the different *Colletotrichum* spp. populations responsible for olive anthracnose in general, and the frequency of occurrence of specific populations in the Algarve

	C. acutatum									*C. gloeosporioides*		Database
	A2-1	A2-2	A2-3	A3	A4-1	A4-2*	A5	A6*	A9**	CG-1	CG-2	reference
A2-1	0.0000	0.0033	0.0017	0.0123	0.0238	0.0256	0.0067	0.0312	0.0042	0.0815	0.0815	AM991131[a]
A2-2	0.0033	0.0000	0.0050	0.0165	0.0238	0.0256	0.0101	0.0313	0.0084	0.0776	0.0776	AM991132[a]
A2-3	0.0017	0.0050	0.0000	0.0144	0.0255	0.0273	0.0084	0.0334	0.0063	0.0834	0.0834	AM991133[a]
A3	0.0123	0.0165	0.0144	0.0000	0.0228	0.0249	0.0082	0.0270	0.0126	0.1014	0.1014	AM991134
A4-1	0.0238	0.0238	0.0255	0.0228	0.0000	0.0017	0.0203	0.0143	0.0277	0.0779	0.0779	AM991135
A4-2	0.0256	0.0256	0.0273	0.0249	0.0017	0.0000	0.0221	0.0164	0.0299	0.0799	0.0799	AM991136
A5	0.0067	0.0101	0.0084	0.0082	0.0203	0.0221	0.0000	0.0270	0.0084	0.0777	0.0777	AM991137
A6	0.0312	0.0313	0.0334	0.0270	0.0143	0.0164	0.0270	0.0000	0.0320	0.0920	0.0920	AJ749700[b]
A9	0.0042	0.0084	0.0063	0.0126	0.0277	0.0299	0.0084	0.0320	0.0000	0.0916	0.0916	DQ991751[c]
CG-1	0.0815	0.0776	0.0834	0.1014	0.0779	0.0799	0.0777	0.0920	0.0916	0.0000	0.0017	AM991138
CG-2	0.0815	0.0776	0.0834	0.1014	0.0779	0.0799	0.0777	0.0920	0.0916	0.0017	0.0000	AM991139
Frequency in Algarve	25%	6%	3%	2%	27%	0%	20%	0%	0%	9%	8%	

[a] sequences for part of the β-tubulin 2 gene: AM992147 (A2-1 and A2-2) and AM992148 (A2-3);
[b] Talhinhas *et al*., 2005;
[c] Whitelaw-Weckert *et al*., 2007;
* A4-2 and A6 are present at Trás-os-Montes, Portugal; ** A9 has been identified in Australia.

Fig. 1. Maps of the Algarve region in Portugal depicting: (a) geographic distribution of olive (shades of grey based on % of total area dedicated to olive cultivation; INE, 2001) at the level of 'freguesia' (the smallest administrative division) and anthracnose pathogens *Colletotrichum acutatum* and *C. gloeosporioides*. Pie-charts represent the proportion of the different genetic entities at each location, while their size reflects the number of isolates analysed over the four years of study. Different genetic entities (*C. acutatum* groups and *C. gloeosporioides*) were found at a single location in different years (A4 and A5 at Pereiro, Olhão; A4 and *C. gloeosporioides* at Monte Branco, Silves), on different host plants in the same year (A2, A3 and *C. gloeosporioides* at Portela de Messines, Silves) and on the same tree or same fruit (A2 and A5 at Santa Margarida, Tavira; A4 and A5 at Monchina, Albufeira; A3 and *C. gloeosporioides* at Portela de Messines, Silves; and A5 and *C. gloeosporioides* at Patacão, Faro), the latter suggests the co-occurrence of different genetic entities on infected fruits; (b) hipsometry (CNA, 1982); (c) soil type (Cardoso *et al*., 1971); and (d) phytogeography (Franco, 1994) and biogeography (Costa *et al.*, 1998) of the Atlantic superprovince(Atlantic-medioeuropean sub-region, Euro-siberian region). Municipalities are ALB – Albufeira, ALC – Alcoutim, ALJ – Aljezur, CM – Castro Marim, FA – Faro, LGA – Lagoa, LGS – Lagos, LO – Loulé, MO – Monchique, OL – Olhão, PO – Portimão, SBA – São Brás de Alportel, SI – Silves, TA – Tavira, VB – Vila do Bispo, VRS – Vila Real de Santo António.

Fig. 2. Patterns of occurrence of olive anthracnose pathogen populations (*C. acutatum* groups A2-A5 and *C. gloeosporioides*, CG) and the related disease severity ratings in the field in the Algarve region during 2004-2007. (a) Temporal variation in the frequency of occurrence of different components of the pathogens; (b) Average anthracnose severity (%) recorded in the field related to the genetic identity of the pathogen populations during autumns 2004-2005 and 2006-2007; and (c) Variation in the frequency of occurrence (%) of different genetic entities of the anthracnose pathogens in relation to host genetic backgrounds.



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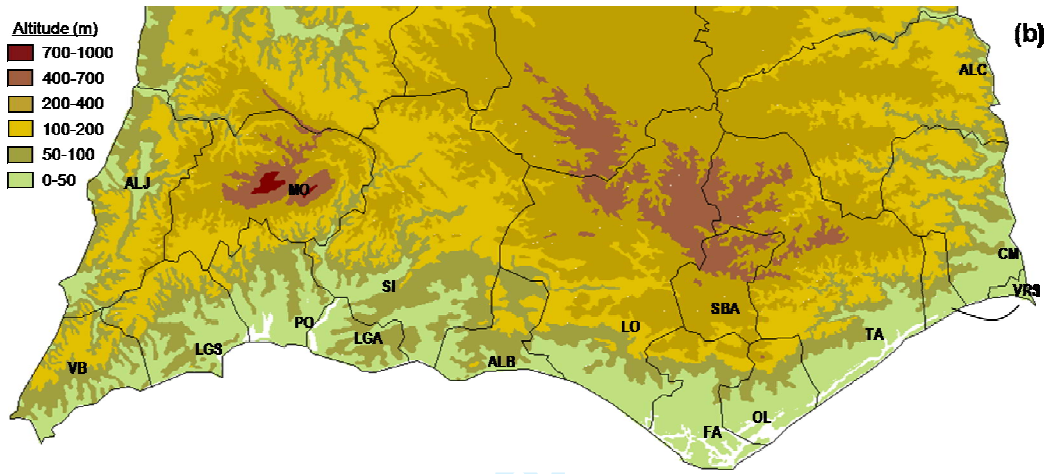

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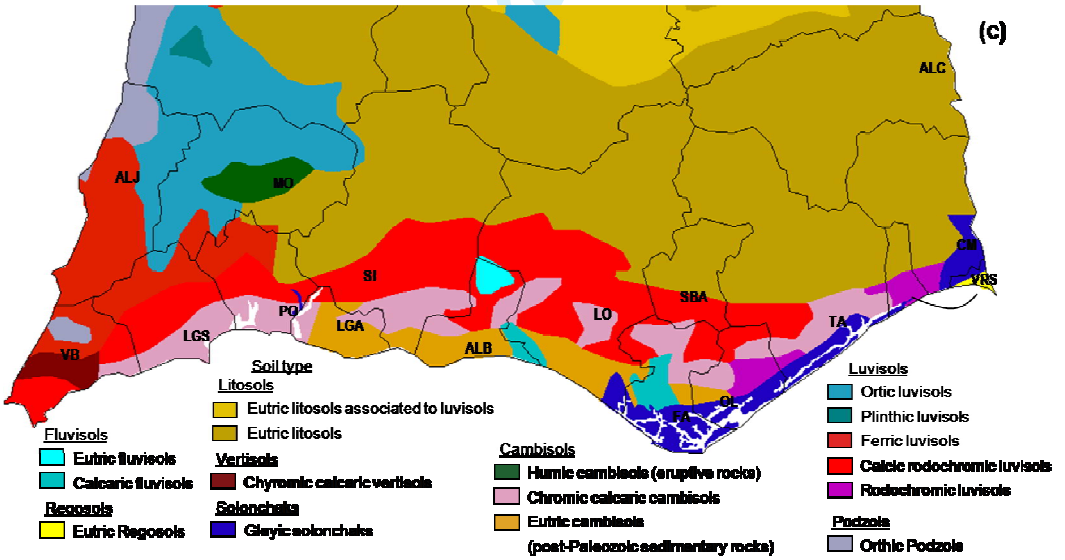

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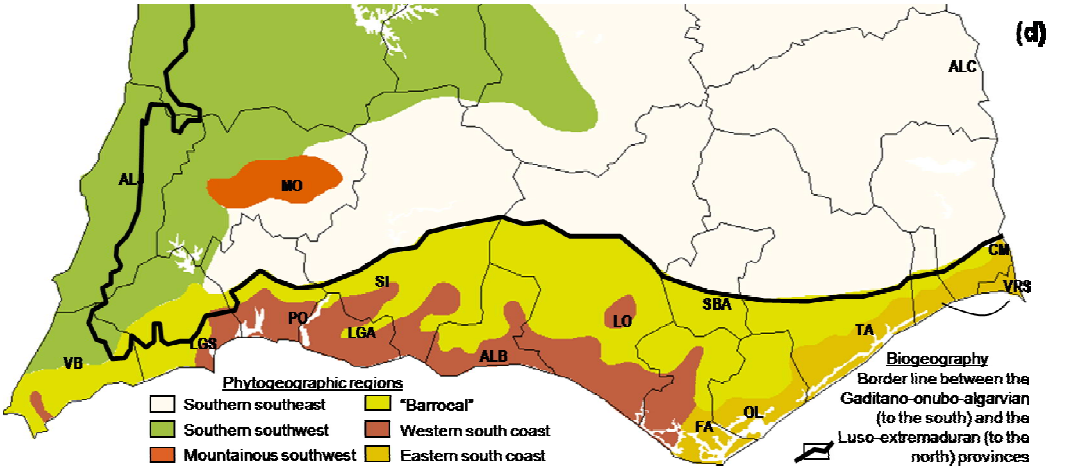

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Fig.1 11

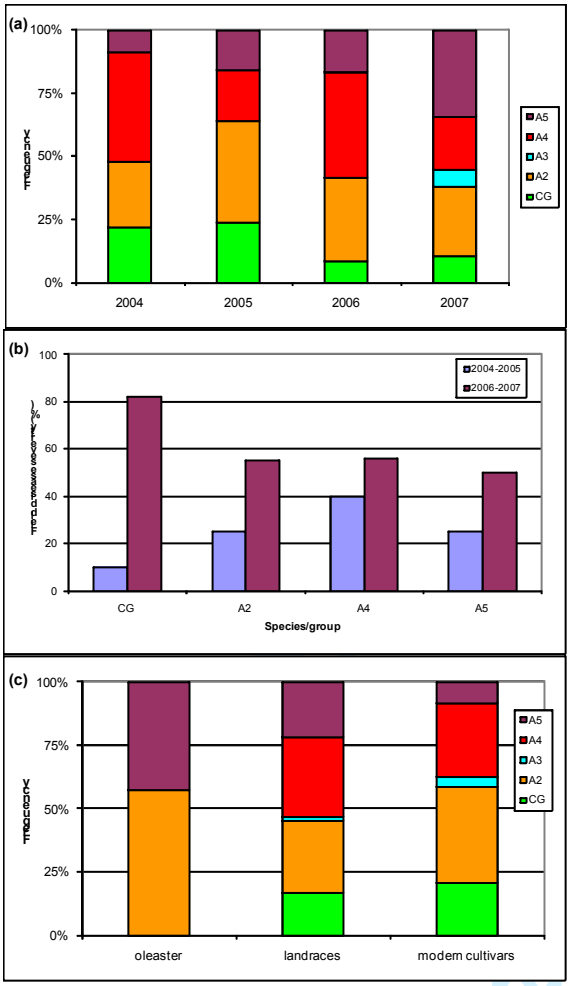

Fig.2 12