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Significant Avocado Diseases Caused by Fungi and Oomycetes

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ABSTRACT

The fruit of the avocado is highly valued not only because of its high nutritional value but also for its role in the cosmetic and health industries. It was used for alimentation as fresh food 9,000 years ago by American communities and later semi-domesticated by Mayan and Aztec civilizations. Nowadays world avocado production is around 3,300 thousand tons harvested from almost 400,000.00 ha. located in tropical and subtropical areas across all continents. Production is increasing and has duplicated over the last 25-years. However, this crop is threatened by notable diseases which could economically limit production and reduce fruit quality. Among them, the disease named Phytophthora root rot caused by the oomycete *Phytophthora cinnamomi* stands out. This destructive invader, which causes extensive losses in agriculture and natural plant communities, is present wherever avocado is cultivated. The present review considers relevant features of the biology and pathogenicity of this plant pathogen of global significance, mainly derived from molecular studies, related to its evolution, population structure and genetic variability, together with current information on management strategies of avocado root rot. Other significant diseases caused by fungi and oomycetes which affect the tree in the field or as postharvest diseases such as branch cankers, fruit rots or anthracnose, are also presented following a "disease profile style", i.e. symptoms, causal agents, epidemiology and control.

Keywords: *Armillaria*, *Botryosphaeria*, *Cercospora*, *Colletotrichum*, *Phytophthora*, *Pythium*, *Rosellinia*, *Sphaceloma*, *Verticillium*
Abbreviations: AM, arbuscular mycorrhizal; **ITS**, internal transcriber spacers; **PCR**, polymerase chain reaction; **PDA**, potato dextrose agar; **PDB**, potato dextrose broth; **RAPD**, random amplified polymorphic DNA; **rDNA**, ribosomal DNA; **RFLP**, restriction fragment length polymorphism; **V8**, eight vegetable juice

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INTRODUCTION

Avocado, *Persea americana* Miller, is a significant fruit crop in tropical and subtropical areas of the planet. Economically, it is the most important species within the family *Lauraceae*, a group among the oldest recorded flowering plants. The genus *Persea* includes about 50 species predominantly of American origin (Bergh 1969). *P. americana* is a tree species apparently originating in a broad area from central and eastern Mexico, through Guatemala to the Pacific coast of Central America. It is a polymorphic species traditionally subdivided into three horticultural races that have recently been recognized as botanical varieties or subspecies: *P. americana* var. *americana* (West Indian race), var. *guatemalensis* (Guatemalan race) and var. *drymifoli*a (Mexican race). These varieties are geographical ecotypes with distinctive adaptations and horticultural and botanical features. Other wild botanical varieties such as var. *nubigena*, var. *steryermakii* or var. *zentmyerii* have also been described recently (Ben-Ya'acov and Barrientos 2003).

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Invited Review

Avocado is an evergreen tree, flowers appear grouped in a compound panicle or raceme and the fruit is a single-seeded berry, highly variable in colour, shape and size among cultivars (Bergh 1969).

Primitive American civilizations initiated the semi-domestication of avocado as a food crop at least 9,000 years ago. Aztecs named this plant as *ahuacalt*, from which the derived Spanish name *aguacate* was used for the first time in 1519 by Martín Férnandez de Enciso in his book "*Summa de Geografía*". The avocado was quickly disseminated from Central America to Spain and the rest of the American continent: Colombia, Venezuela, Ecuador to Peru. Finally, during the XIXth century its presence in all continents was complete.

The avocado fruit has excellent nutritional value. The Australian Heart Foundation has certified this fruit as a "heart-healthy" food, recommending avocado as a dietary source of fat. It contains a high oil content (3-30%, depending on the variety) and constitutes a supply of proteins, carbohydrates minerals and vitamins. Avocado oils are also highly valued in the cosmetic industry (Knight 2002). World avocado production is around 3,317 thousand tons harvested from 388,101.00 ha (FAOSTAT 2007). Mexico is the main avocado producer in the world (1,137 thousand tons), followed by the USA (247,000 tons) and Indonesia (227,000 tons). World avocado production has doubled over the last 25-years (1981-2006). The highest rate of increase during this period has occurred in New Zealand (95-fold), followed by Australia (16-fold) and Morocco (16-fold). Nowadays, avocado is cultivated in more than 60 countries throughout all continents and it seems that avocado production will continue to develop, especially in areas such as China, with 90,000 tons reported in 2006 (FAOSTAT 2007). European countries such as France, Germany and Great Britain are the principal importers, consuming more than 35% of world avocado production.

However, the avocado crop suffers notable diseases which could affect production and limit its development. Because of the enormous quantity of pathogenic microorganisms and abiotic factors that may affect the tree, this review is focused on the most important biogenic diseases of avocado from the point of view both of their potential to cause important economic losses, and their worldwide distribution. Among these, Phytophthora root rot caused by the oomycete *Phytophthora cinnamomi* Rands is without doubt the most serious disease of avocado limiting the avocado industry in nearly every country where it is grown. Therefore, this work is arranged into two parts. In the first section, the most important issues relating to the biology of *P. cinnamomi*, its pathogenicity to avocados, together with the principal methods being applied to control avocado root rot are reviewed. In the second section, other important diseases caused by fungi and other oomycetes are outlined as "disease profiles", considering the most significant issues related to their distribution, causal agents, epidemiology and the main control measures.

PHYTOPHTHORA ROOT ROT

Phytophthora cinnamomi Rands is an important oomycete soilborne pathogen with a global geographical distribution which infects a large number of plants in agricultural, horticultural and forest ecosystems. Rands first reported the pathogen in 1922 from a cinnamon tree (*Cinnamomum burmannii* Blume) in Sumatra. Since then, *P. cinnamomi* has been recognized extensively around the world, being cited in more than 75 countries. In general, *P. cinnamomi* is located in warm, tropical and subtropical areas of the world (CABI 1991). There are more than 3,000 susceptible plants described. Of these, one third have worldwide distribution whilst the rest are localised in Australasian natural forest eco-systems (Zentmyer 1980; Weste *et al.* 2002). It has been confirmed that in eucalyptus forests in Western Australia, the dieback of susceptible eucalyptus trees caused by *P. cinnamomi* leads to shifts in plant and animal communities, both above and below ground (Weste *et al.* 2002). Within the genus *Phytophthora*, *P. cinnamomi* is the most dispersed, and with a wider range of hosts, is considered as an invasive species and one of the most destructive plant pathogens which threaten native biodiversity (Zentmyer 1983; Bohlen 2006; http://www.issg.org).

In avocados, *P. cinnamomi* causes a disease named Phytophthora root rot. Symptoms of decline on avocados were first described in the 1920s in a number of different countries and they were initially associated with high soil humidity. However, the isolation of *P. cinnamomi* from declined-avocado roots in Puerto Rico confirmed that the pathogen was the causal agent of the decline-symptoms (Tucker 1929). During the 1940s, different authors confirmed that *P. cinnamomi* was the primary factor in the decline of avocado trees and that water excess assists pathogen development (Wager 1940). It was also established that water is an important factor for formation, dispersal and germination of spores. Extensive research on avocado root rot caused by *P. cinnamomi* was carried out at the University of California (Riverside), in the decades that followed, with Dr. George Zentmyer being one of the most significant researchers of this pathogen and its associated diseases. Subsequently, the pathogen has been detected in all producer countries.

Nowadays, Phytophthora root rot is the main disease affecting avocados across all continents around the world causing severe losses in fruit production (Zentmyer 1980). In Mexico, where the disease is known as "*la tristeza del aguacate*" (the sadness of the avocado), the pathogen is present in all the principal avocado producing areas, with incidences varying between 5-90%, depending on the area, and increasing during the last few years (Téliz 2000). In California, the number of infested orchards in some areas reached ranges between 60-90% causing significant economic loss (Coffey 1989). In Eastern Australia, the pathogen is widely distributed affecting avocado production (Pegg *et al.* 1987). In South Africa, where the avocado crop is relatively recent, the number of infected orchards has increased steadily since their establishment, affecting about 20% of all trees (Milne and Chamberlain 1971). In the Andalusia Region (Spain), 40% of decline-symptomatic orchards are infested by *P. cinnamomi* (Pérez-Jiménez *et al*. 2005). In Israel, the pathogen was first isolated in 1982 and throughout the following years the number of infested groves increased (Baum and Pinkas 1988).

Biology of *Phytophthora cinnamomi*

Taxonomy and phylogenetic studies

The established taxonomy of the Peronosporomycetes (oomycetes) is changing, especially after the incorporation of modern molecular tools for phylogenetic studies, which are mainly based on analyses of small ribosomal subunit RNA and different protein genes. Structural and biochemical features differentiate oomycetes from true fungi and, nowadays, they are grouped with other taxa in an assemblage called the Stramenopiles (http://tolweb.org). The common ultrastructural features of Stramenopiles are mitochondria with tubular cristae and flagella with tripartite hairs. Phylogenetically, the Stramenopiles are much closer to protists such as Alveolates (e.g. cilliates or apicomplexa) than they are to fungi. Other taxa in the diversified Stramenopiles group included algae (Heterokonts or Chrysophytes) and non-algal protists. It is argued that they diverged from other lineages of the "crown" of eukaryotic trees one billion years ago (Sogin and Silberman 1998; Baldauf *et al.* 2000). Moreover, molecular phylogenetic studies within oomycetes are giving rise to important evolutionary and taxonomic consequences. Thus, results of extensive recent phylogenetic analysis of oomycetes suggests that the genus *Phytophthora* should be transferred from Pythiaceae to Peronosporaceae, as there is evidence for monophyly of a clade comprising *Phytophthora* and the obligate biotrophs Peronosporales (Reithmüller *et al.* 2002; Kroon *et al.* 2004; Göker *et al*. 2007).

Within the genus *Phytophthora*, comparisons of DNA sequences have improved the understanding of species relationships and evolution. Traditional classification systems of *Phytophthora* had mainly been based on morphology of the sporangium and gametangia, and the genus was divided into six main groups, *P. cinnamomi* belongs to group VI (Waterhouse 1963; Ho 1981; Stamps *et al.* 1990). Others criteria such as temperature growth range, number and size of chromosomes, protein patterns or pathogenicity tests on different hosts have also been used for species classification (Erwin 1983; Oudemans and Coffey 1991). Molecular phylogenetic studies achieved with different DNA markers grouped *Phytophthora* species into eight clades and confirmed that traditional morphological groupings were not natural assemblages (Lee and Taylor 1992; Cooke and Duncan 1997, 2000b; Föster *et al.* 2000; Kroon *et al.* 2004). Biochemical probes and molecular biology techniques are also improving the methods for the identification of *P. cinnamomi* and other *Phytophthora* species (further described in Diagnosis section).

Geographic origin, population structure and genetic variability of Phytophthora cinnamomi

The geographic origin of *P. cinnamomi* has been widely discussed. Different "classical" considerations, such as the analysis of the mating type frequencies of the pathogen in an area (*P. cinnamomi* is heterothallic with two mating types: A1 and A2), its coexistence with native resistant plants, or its morphological and biochemical diversity, allowed different authors to hypothesise about the geographic origin of *P. cinnamomi.* Ko *et al*. (1978) identified Taiwan as a possible centre of origin, and von Broembsen and Kruger (1985) proposed the existence of another potential centre of origin in South Africa. Later, Zentmyer (1988) suggested a possible centre of origin for the pathogen as southern Asia, an area covering Sumatra, Malaysia and Taiwan, and also New Guinea. Moreover, analysis of genetic variation of populations of *P. cinnamomi* have highlighted some important aspects, not only related to possible origin and distribution of *P. cinnamomi*, but also to host-specialisation, pathogen aggressiveness or reproduction mechanisms as sources of variability among others.

Isozyme studies on *P. cinnamomi* populations from different regions of the world have revealed that, in general in most areas, genetic and genotypic diversity among isolates is low (Old *et al.* 1984, 1988; Oudemans and Coffey 1991; Linde *et al.* 1997). In particular, in Australasian and South African populations, isozyme analysis indicated that low levels of space-temporal genetic diversity, higher frequency of A2 mating type and the absence of sexual reproduction, are common features among *P. cinnamomi* isolates (Old *et al.* 1984, 1988; Linde *et al.* 1997). Meanwhile, isolates from Papua New Guinea, the proposed centre of origin of the species, show high genotypic diversity, especially in A1 isolates (Old *et al.* 1984). Chang *et al*. (1996) evaluated the amount of genetic variation in a Taiwanese population using random amplified polymorphic DNA (RAPD) technique and identified markers of compatibility groups and markers of avocado isolates. They also found that in small areas with both mating types present no hydridization occurs. Finally they considered the possible existence of host-specified races in *P. cinnamomi*. Linde *et al*. (1999a) assessed genetic differentiation between Australasian and South African populations using restriction fragment length polymorphism (RFLP) and RAPD techniques and confirmed previous results finding a similar structure and close relationships between both populations. Further, Dobrowolski *et al*. (2002, 2003) developed four microsatellite loci from *P. cinnamomi* and analysed a large collection of *P. cinnamomi* isolates from Australia and other parts of the world. They identified three microsatellite multilocus genotype groups and considered that they represented three clonal lineages of *P. cinnamomi*, which have spread in most regions. These authors argued that observed mitotic recombination events may explain pathogenic variability observed within the clonal lineages of *P. cinnamomi*. With regard to genetic variability, it is important to point out that isolates of *P. cinnamomi* from all over the world and from many different hosts have similar growth patterns, morphology and physiological behaviour, although some differences, mainly between both mating types, have been recorded (Zentmyer 1980). However, a considerable ability to produce a range of pathogenic phenotypes had been broadly recognised in this species (diversity in pathogenesis is further described in the Pathogenicity section).

All these results corroborate early reports that *P. cinnamomi* has been introduced in relatively recent times in different areas from its place of origin, possibly Papua New Guinea (Shepherd 1975; Old *et al.* 1984; Zentmyer 1988; Linde *et al.* 1997). The spread of *P. cinnamomi* from the possible centre or centres of origin to other continents (America, Australia, West Europe and Africa) could have been caused by European or Malaysians explorers during transportation of soil and vegetal material. Locally, movements of soil, water, infected nursery material and plants have favoured the dispersal of *P. cinnamomi*.

Hosts of Phytophthora cinnamomi

In the years following the identification of *P. cinnamomi* (Rands 1922) the pathogen was isolated from many different plants. During the fifties the number of hosts increased notably, especially on plants from warm and tropical areas of the planet, but the great destructive potential of *P. cinnamomi* only became apparent during the 1970s, hence research was notably increased during this period. The most significant food-crop losses due to *P. cinnamomi* root rot occur in avocado. In natural ecosystems it has caused extensive damage in jarrah (*Eucalyptus* spp.) forests in Australia (Weste *et al.* 2002). However, among the hosts of *P. cinnamomi* listed by Zentmyer (1980), woody plants stand out, including significant economically important crops and forest plants such as: beech (*Fagus* spp.), chesnut (*Castanea dentata* and *C. sativa*), *Cinchona* spp., *Cinnamomum* spp., cypress (*Chamaecyparis lawsoniana*), fir (*Abies* spp.), *Juglans* spp., oak (*Quercus* spp.), pineapple (*Ananas comosus*), *Pinus* spp., *Prunus* spp., *Rhododendron* spp., *Vaccinum* spp. and many other ornamental trees and shrubs.

Life cycle of Phytophthora cinnamomi

P. cinnamomi is a hermaphrodite species regarded as heterothallic, with two mating types designated as A1 and A2. The common form with worldwide distribution is the A2 type, whilst the A1 type is geographically limited with fewer hosts. Although a general similarity of both mating types exists, differences in phenotypic features and genotype have been widely recognized (Shepherd *et al.* 1974; Zentmyer 1980; Old *et al.* 1984; Oudemans and Coffey 1991; Chang *et al.* 1996; Linde *et al.* 1997, 1999; Dobrowolsky *et al.* 2003). The life cycle of *P. cinnamomi* is represented in **Fig. 1**.

The sexual stage of *P. cinnamomi* can be activated through inter- and intraspecific crosses on culture medium when an isolate of *P. cinnamomi* is crossed with the opposite mating type (an isolate of the same or other different species of *Phytophthora*). Different studies manifest that heterothallic *Phytophthora* spp. can be considered as homothallic, but they are dependent on hormones α^1 or α^2 (molecular weights between 100 and 500) produced by the opposite mating type for induction of sexual reproduction (Ko 1988). Otherwise, in the real homothallic species, this external induction is not necessary for sexual reproduction. In this context, Ko (1988) proposed the term "type of induction" instead of the mating type or compatibility type. Moreover, this author reported a process of reversible conversion of induction types in several *Phytophthora* species with specific chemicals such as the fungicides chloroneb and ethazol. The sexual stage of *P. cinnamomi* could also be

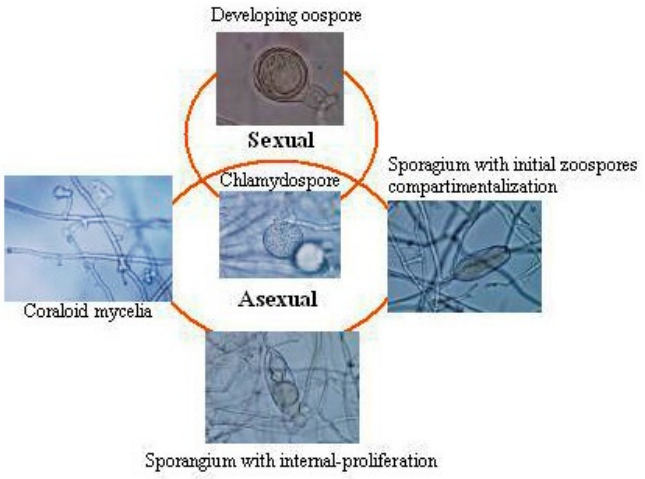

Fig. 1 Representation of the life cycle of *Phytophthora cinnamomi*. Reproductive structures of the pathogen related to its vegetative, sexual and asexaul life cycle phases are represented.

induced through initiation of homothalic behaviour, producing selfing oospores, as has been observed in aged single cultures, adding avocado root extracts to the medium (Zentmyer 1979, 1983), in the presence of the fungus *Trichoderma* (Brasier 1975), or *in planta* as recently described by Jayasekera *et al*. (2007). These different stimuli have been related to host defence mechanisms and survival of the pathogen (Zentmyer 1979; Jayasekera *et al*. 2007).

P. cinnamomi is a diploid organism in the vegetative state. Meiosis occurs in the gametangia, thus it is haploid only in gametangia prior to fertilization. The estimated n chromosome number is 9 or 10 (Brasier and Sansome 1975). Gametangial interaction and further development of the oospore in *Phytophthora* species was described by Hemmes (1983). When oogonial and antheridial initials are in contact, the oogonial initial penetrates the antheridium until it emerges at the opposite side. Subsequently, functional cytoplasm flows along the oogonial stalk which is later tapped. Afterwards, the cell wall of the oogonium becomes thick and all the haploid nuclei, except one, migrate to the periphery. A fertilization tube originates from the antheridium, perforates the common wall between the antheridium and the oogonial stalk, travels up the oogonial stalk, and penetrates the oosphere wall leaving its nucleus. Following nuclear fusion, a membrane delimiting the oosphere is formed. Oogonia of *P. cinnamomi* have a diameter of 23–41 (31.9) µm, with a smooth wall, which becomes yellow or golden with age. Antheridia are mostly unicellular and amphigynous although some are paragynous, with 8–29 µm length and 12–25 µm width. The oospore is spherical almost plerotic with 22–36 (28.1) µm diameter (Waterhouse and Waterson 1966a; Ho and Zentmyer 1977a; Gerrettson-Cornell 1983; López-Herrera and Pérez-Jiménez 1995; Daniel *et al.* 2003; http://www.phytophthoradb.org).

P. cinnamomi sporangia are formed only in aqueous solutions. Their size and shape vary depending on environmental conditions and nutritional status in which they develop (Ribeiro 1978). Therefore, this is not a very reliable characteristic for identification. Sporangia can germinate by the formation of a germination tube (direct germination) or by the liberation of zoospores (indirect germination). Physical factors, such as the temperature of the liquid where they formed, determine whether germination is direct or indirect. Zoospores are formed by compartmentalization of the mature multinucleate sporangium (Hyde *et al.* 1991). Each sporangium liberate from 10 to 30 motile zoopores. Internal proliferation, which means the formation of new sporangia, could occur inside the empty sporangia –by expansion of sporangiophore cytoplasm across the basal septum– or by development of the sporangiophore across the empty sporangia, originating a new sporangia at some distance from the original sporangium. The sporangia have thin sporrangiophores (3 µm), sometimes branched. They are ovoid or ellipsoid 14–83 × 13–43 (36.9 × 25.5) µm, non-papilate with a thin apical engrossment (Waterhouse and Waterson 1966a; Ho and Zentmyer 1977a; Gerrettson-Cornell 1983; López-Herrera and Pérez-Jiménez 1995; Daniel *et al.* 2003; http://www.phytophthoradb.org).

P. cinnamomi zoospores are similar to those of other *Phytophthora* and *Pythium* species. They are ovoid in shape, with a longitudinal ventral groove which gives them a dorsi-ventral polarity. They have mobility, with two flagella which expand from this ventral groove; the tinsel flagellum forward and the long whiplash behind. Most of them are uninucleate (Shepherd and Pratt 1974) with a size of 20 × 12 µm (Hardham 1987; Hyde *et al.* 1991). After their release to the medium, they form cysts by engrossing their cell wall into a spherical shape (8-15 µm). Usually they germinate directly through the formation of a germination tube, which forms an apressorium or a uninucleate microsporangium. Sometimes they can germinate indirectly, developing a secondary zoospore like the original one. The type of germination depends on physical factors and the internal metabolism and could influence the pathogenic behaviour of *P. cinnamomi* (Ho and Zentmyer 1977a).

Chlamydospores were described by Rands (1922) as thin wall structures, globoses or pyriform, terminal or in lateral branches, forming frequently a cluster or bunch with 3-10 spores, with a diameter of 16–43 (27.1) µm, cell wall (max. 0.6 µm). These spores have a high rate of lipid-like bodies, peripheral vesicles and small vacuoles (Hemmes and Wong 1975). They are resistant and can persist in soil or infected tissues for long periods of time. Chlamydospore germination occurs directly by formation of a germ tube with numerous vesicles at the tips. Mycellium of *P. cinnamomi* is coralloid and presents typical swollen hyphae.

Effect of environmental factors on Phytophthora cinnamomi development

P. cinnamomi tolerate moderate temperatures. *In vitro* studies related to maximum, optimum and minimum growth temperatures of *P. cinnamomi* revealed that the optimum temperature for mycelial growth is around 24 and 27°C and that it does not grow below 10°C or above 33-34°C. These studies also indicate that there is no correlation between the geographic or host origin of the isolates and the growth response to different temperatures (Shepherd and Pratt 1974; Zentmyer *et al.* 1976a; Zentmyer 1981). Mycelium of *P. cinnamomi* developed on potato dextrose broth (PDB) cannot survive after treatments at 36°C lasting 2-3 days, at 39°C for 1-2 h or at 45°C for 10-30 min. Regardless of the effect of low temperatures on *P. cinnamomi*, it is known that the pathogen cannot survive on the soil surface at less than 0°C. Otherwise in these soils it can survive at a depth of 10 cm, where the temperature is above 0°C (1-2.5 °C) (Benson 1982). In controlled experiments the pathogen survived in the soil for 12 days at –2°C, but not for 20 days at that temperature (Zentmyer 1980).

In general terms, *P. cinnamomi* grows well *in vitro* with a pH value between 4.5 and 5.5 in the presence of arginine as a N source (Cameron and Milbrath 1965). Chlamydospores germinate over a range from pH 3.0 to 9.0, with an optimum at pH from 5.0 to 7.0 (Mircetich *et al.* 1968). Sporangia production is elevated over a range from pH 4.0 to 7.0 (Chee and Newhook 1965) while it is nil with a pH value less than 3.5 (Benson 1984).

Phytophthora species tolerate well different levels of O_2 and CO_2. Mycelium of *P. cinnamomi* grows and infects at normal air or at low O_2 (0.05-1.5%) plus elevated CO_2 (10-20%) (Mitchell and Zentmyer 1971a). However, sporangia production by *Phythopthora* species is reduced under lower levels of O_2 and greater levels of CO_2 than in normal air (Mitchell and Zentmyer 1971b). Oospore production by *P. cinnamomi* is favoured by low levels of O_2 (1-5%) but decreased with elevated levels of CO_2 (5-15%) (Mitchell and

Zentmyer 1971b).

Regardless of the osmotic water potential, the optimum for *P. cinnamomi* growth *in vitro* is between -10 to -15 bars. The pathogen grows best in liquid medium with osmotic water potential of -2 to -8 bars and is insignificant at -20 and -30 bars. In soil, chlamydospore production occurs at different moisture levels, contrary to the sporangia which need saturated soils for their production (Reeves 1975). For sporangial production the optimun soil matric potential ranges between -15 and -25 mbars (Benson 1984). Zoospore movement is conditioned by the soil type and also by the osmotic water potential. The movement of the zoospores is helical, needing a space for mobility of 50-140 µm, thus free movements is difficult through soil pores less than about 190 µm in diameter. Besides, high matric potential favours large soil pores filled with water, which are optimum for zoospores swimming (Zentmyer 1980).

Originally it was thought that *P. cinnamomi* formed sporangia only when cultures were incubated under continuous light or under alternate light/dark. However, later studies performed with axenic cultures and different monochromatic light demonstrated that sporangia production by *P. cinnamomi* is light-variable, but not light-dependent. They are formed under darkness and under different wavelengths ranging from the near-UV (312 nm) to infrared (1,300 nm) (Zentmyer and Ribeiro 1977).

Nutritional requirements, saprotrophic behaviour and survival

Favourable carbon sources for *P. cinnamomi* growth on synthetic media are D-glucose, D-fructose, D-mannose, and D-xylose. KNO_3 is a good nitrogen source (Roncadori 1965; Zentmyer 1980). In nonsterile soil extract, the best carbon sources for *P. cinnamomi* growth and sporangium production are dextrin, starch and sucrose. Good organic nitrogen sources for growth are glutamine and glutamic acid. The production of sporangia is positive with all amino acids in the presence of $Ca(NO_3)_2$. Under axenic conditions and in nonsterile soil solution, glucose and glutamic acid inhibit sporangia production (Zentmyer 1980). Chlamydospores and oospores are abundantly formed on eight vegetable juice (V8) broth, but not on PDB. Carrot agar and V8 agar are the most appropriate media for oospore production. In soil, germination of chlamydospores is stimulated by root exudates containing sugars and amino acids (Zentmyer 1980).

Thiamine (vitamin B_1) is the only vitamin required by *P. cinnamomi*. The pathogen can absorb and accumulate thiamine from the medium, but it can neither absorb nor synthesise it from thiazole or pyrimidine (Zentmyer 1980). *Phytophthora* and *Pythium* species cannot synthesise sterols. They are necessary for production of oospores and sporrangia but not for mycelial growth (Hendrix 1965). Mycelial growth of *P. cinnamomi* can be increased by adding Ca^{2+} to the medium, meanwhile sporangium production is inhibited with Ca^{2+} (at 1,000 mM) and Fe^{3+} (at 10 mM) (Zentmyer 1980).

P. cinnamomi is not an aggressive saprophyte but it has competitive saprophytic ability, especially under high humidity conditions, invading organic matter and soil in the presence of other microorganisms. The pathogen can colonize fresh organic matter and fresh or rotted roots in competition with other soil microorganims. In the absence of fresh roots or organic matter, the mycelium lyses and the pathogen forms resistance structures, chlamydospores and sporangia, in a very short period of time (Reeves 1975). In general, *P. cinnamomi* is concentrated around living roots and is more difficult to isolate in advanced stages of the disease or in bare soil (Marks *et al*. 1975). In the absence of a host, *P. cinnamomi* can survive up to six years in humid sandy loam soil maintained at 20°C. Conversely, when the soil is dried to 2-3% moisture content, it survives only three months (Zentmyer and Mircetich 1966).

Pathogenicity

Symptoms of Phytophthora root rot of avocados

Generally, the primary symptom caused by *P. cinnamomi* is root rot; avocado feeder roots present necrotic lesions, the pathogen progresses penetrating the epidermis and cortex, killing the underling tissues and invading the entire feeder root system (Zentmyer 1980; Phillips 1993). In advanced stages of the disease, only scarce small roots, blackened and brittle, are present in the soil. *P. cinnamomi* does not normally progress from feeder roots into large woody roots (Zentmyer 1980). Secondary symptoms, resembling those of drought, are a consequence of reduced uptake of water and nutrients. Plants become chlorotic, with smaller leaves than normal, which wilt frequently and drop prematurely, new growth is limited, giving diseased trees a sparse appearance (**Fig. 2**). Occasionally, cankers are found on large roots and trunk cankers, with white exudates may occur at the soil level (Crandall 1948). Large numbers of small fruits may be commonly observed on infected trees. Fruits could also be rotted by water splash or contact with infected soils. Severely affected trees eventually die.

Disease development. Zoospores released from the sporangia move toward the infection sites through the action of the two morphologically different flagella which emerge form the ventral longitudinal groove. Pathogen zoospores are attracted to the root elongation zone of the avocado, behind the root tip apex, in response to chemical signals (amino acids, sugars or ethanol) (Zentmyer 1980). Electrical signals generated in the rhizosphere as a consequence of electrogenic ion transport at the root surface have also been confirmed to mediate in tactic responses of zoospores (van West *et al*. 2002). At the root surface, before they cyst, zoospores orientate with their ventral surface facing the root, the tinsel flagellum forward and the long whiplash behind. The time between alignment and encystment is usually 9-14 s. During encystment (30 seconds to two minutes), the two flagella are lost and two different types of small peripheral vesicles (0.3 µm in diameter), which are differentially located at the dorsal and ventral surface of the zoospore, secrete their contents on the outside of the developing cysts. The content of the dorsal vesicles is secreted to form a mucilage-like coating over the surface of the cysts and the content of the ventral vesicles forms a pad of adhesive material between the cyst and the root surface (Hardham and Gubler 1990). Twenty minutes after attachment, the spores germinate; the germ tube emerges from the ventral surface and grows straight towards the root. The emergent hyphae either penetrate into the avocado roots directly or after forming an appresorium-like swelling (Aveling and Rijkenberg 1989). Within 24 hours, necrotic lesions in the elongation zone are present, spreading fast throughout the small feeder roots. After 48 hours, mycelium of *P. cinnamomi* may be observed in the cortex, parenchyma cells are collapsed and numerous microsporangia are present on the root surface. Within 4-6 days hyphae have proliferated all over the cortex and stele forming abundant swellings and hyphal vesicles in the cor-

Fig. 2 Avocado tree expressing aboveground symptoms of Phytophthora root rot (A). Feeder roots of avocado; white-healthy roots and black-necrotic roots infected by *Phytophthora cinnamomi* (**B**).

tex. Usually, *P. cinnamomi* only invade the small feeder root, however, under special conditions, it could grow and infect secondary roots. On trunk, branches and large roots, *P. cinnamomi* can causes cankers, which are initiated on wounds, usually associated with an excess of water. In cankers, the pathogen grows into the outer vascular system from the cortex moving faster vertically than horizontally in the woody host (Zentmyer 1980).

Physiology of the disease

Physiological and biochemical features of *P. cinnamomi* infections are poorly understood despite the economic and ecological importance of this species. There is a direct relation between the destruction of feeder roots and the limited nutrient and water uptake and the progressive decline of the trees or aboveground systems. *P. cinnamomi*, as in pathogenic fungi, penetrates host cell walls through enzymatic action and mechanical pressure, direct on host cells or by growing among intercellular spaces. In fact, the penetration occurs mainly in the elongation zone, which presents a low mechanical resistance to penetration, with a young, thin-walled epidermis, an undifferentiated exodermis and an unthickened endodermis (Phillips 1993). Thus, host penetration involves the production and secretion of different enzymes which digest and degrade the plant cell wall. Borrod (1974) studied the pathosystem *P. cinnamomi* - *C. sativa* and established that the pathogen synthesizes a cellulase in the presence of cellulose and glucose and also described pectolytic activity. Casares *et al.* (1986) detected *in vitro* the capacity of *P. cinnamomi* to dephenolize lignin and to oxidize some monophenols and di- and tri-OH phenols with *o*-dihydro structure such as phenol, pyrocatechol or gallic acid at a concentration of 10^{-3} M on malt extract agar. More recently, Götesson *et al*. (2002) have demonstrated that in *P. cinnamomi*, polygalacturonases, which are believed to be important pathogenicity factors, are encoded by a large multigene family consisting of more than 20 genes. They also confirmed a closer relationship with polygalacturonases from true fungi than those from plants.

Additionally, *Phytophthora* species synthesize a group of proteins called elicitins. All *Phytophthora* elicitins are 10 kDa proteins comprised of 98 amino acids which induce in several plant species symptoms closely resembling the hypersensitive response; leaf necrosis, accumulation of pathogen-related mRNAs and proteins, and increased protection against pathogens (Nespoulus *et al.* 1992). *P. cinnamomi* produces two classes of elicitins with differet physico-chemical features: α-cinnamomin (acidic) and β-cinnamomin (basic) (Pernollet *et al.* 1993). Ivanova *et al.* (2002) indicated that, in cranberry, cinnamomin might function as a toxin, capable of inducing ageing and cell death. There is no clear proof related to the role of different toxins in the infection process of *P. cinnamomi*; however, some authors identified in the cytoplasm of this pathogen, and other species of the genus, the presence of mycolaminaran (β-1,3-glucan, β 1-6 branched). It is thought that mycolaminarans act as intercellular reserves of carbohydrates and also that they could act as elicitors (Yoshikawa *et al.* 1983). Zentmyer (1980) reported that mycolaminarans and complex glucans from the cell wall of *P. cinnamomi* induced wilting in *Persea indica.* This response could be the result of a toxic effect or a defence response. Furthermore, some carbohydrate polymers could be related to wilt symptoms and water stress. The recent sequencing of the *P. sojae* and *P. ramorum* genomes reveals a complex scenario in which a rapid expansion and diversification of many protein families associated with plant infection occurs in these *Phytophthora* species (Tyler *et al*. 2006). Gene products of infection-related genes in these significant pathogens include hydrolases (such as proteases, glycosyl hydrolases, pectinases, cutinases, chitinases, lipases, phospholipases), protease inhibitors, protein toxins, secondary metabolite biosysthesis, ABC transporters and effectors. Effectors comprise elicitins, elicitin-like and the Avh (aviulence homolog) family; a diverse family of about 350 infection-associated genes. These genes are similar to previously identified "avirulence" or "effector" genes in several oomycetes, which interact genetically with plant disease resistance genes that encode defence receptors (Deng 2006; Xiao 2006).

Pathogenic variability

As previously observed, *P. cinnamomi* has considerable ability to produce a range of pathogenic phenotypes. As no evidence of sexual reproduction (genomic recombination) has been found in the field, it is supposed that variation in pathogenesis characters must be arising asexually. Early studies reported a series of disease-associated phenotypes among isolates of *P. cinnamomi* from different hosts. Further, variations in pathogenesis, physiology and inoculum production have been broadly recognized. Thus, Rands (1922) differentiated two "strains" of *P. cinnamomi*, one more virulent on cinnamon than the other. After that, terms such as "physiologic strains" (Crandall *et al.* 1945), "physiological races" (Torgenson 1954) or "biotypes of the pathogen" (Manning and Crossan 1966) have been used to refer to the existence of significant different capacity between isolates to induce symptoms on different hosts or the existence of differences in virulence on the same hosts. Zentmyer and Guillemet (1981) provided additional evidence for strains or biotypes of *P. cinnamomi* based on different host specificity. Dudzinsky *et al*. (1993) detected variations in the amount of disease caused by Australian isolates to *Eucalyptus* spp. and confirmed that these variations were not related to mating type or isozyme genotype. Pérez-Jiménez (1997) identified significant differences in virulence within Spanish isolates of *P. cinnamomi* from avocado. Robin and Desprez-Loustau (1998) provided evidence of variable levels of virulence within isolates from *Quercus* spp., again not related to mating type or electrophoretic type or to the age of cultures, and confirmed the absence of host specialisation in this species. Linde *et al*. (1999b) confirmed non-host specialisation and distinguished differences in virulence among South African isolates. They found a positive correlation between growth rate of isolates *in vitro* and level of pathogenicity, whereas age of cultures correlates negatively. Finally, they also detected different levels of pathogenicity for different multilocus isozyme genotypes.

Environment and disease development

In a system as complex as soil, with multiple interactions amongst pathogens, active growing plants, and an enormous diversity of microorganisms, it is difficult to determine which environmental factors are the most important and what is the specific action of each of them (Zentmyer 1980). Development of root rot caused by *P. cinnamomi*, like that caused by other pathogens, depends on the pathogen-population density. Under natural conditions, there are great seasonal variations of *P. cinnamomi* soil populations which correlates first with soil temperature and then, under conductive temperatures, with soil water potential (Weste 1983). There exists a direct relationship between temperatures required for growth and production of sporangia and the temperatures favourable for the disease to progress. Infections occur in a range of 15-27°C, with the optimum at 22-26°C (Zentmyer 1981). In general, with soil temperatures below 10°C the population of the pathogen in the soil is limited, likewise under low water potential, even under optimum temperature, the pathogen cannot survive outside the host (Zentmyer 1980).

On the other hand, the relationship between disease development and an excess of water in the soil, normally associated with poor drainage, has been recognized since initial studies of root rot (Rands 1922). The disease can also progress in well-drained soils, under frequent rain conditions or in especially rainy years (Zentmyer 1980). Production of sporangia is controlled by soil water suction pressure rather than absolute water content (Reeves 1975; Sterne *et*

al. 1977; Benson 1984). Thus, under the same water content, in sandy soils the production of sporangia will be greater than in a clay soil, where soil water suction pressure is higher. It is not clear if under high humidity conditions the severity of the disease is increased by a consequence of a chemical modification of the soil, a reduction of the host resistance, an increase of pathogen activity or a combination of all of these. Infections caused by *P. cinnamomi* accelerate exponentially under excess water. This indicates that zoospores are the primary agent in disease initiation and that soil water is necessary for the production of sporangia and for zoospores to be released and move towards the roots (Zentmyer 1980).

In relation to pH, root rot caused by *P. cinnamomi* develops in a range of 3.2–7.0, the optimum for the disease being pH 6. With values below 3 or above 8 the disease does not progress (Crandall *et al*. 1945; Zentmyer 1980). *In vitro*, at these extreme values, the production of sporangia and zoospores is nil. In the field, severe symptoms are observed in soils with pH between 4.5 and 7.5 (Bingham and Zentmyer 1954). In relation to soil O_2 content, it has been observed that under low levels of O_2, the efficiency of *P. cinnamomi* to infect the avocado roots is limited (Curtis and Zentmyer 1949). A poor aeration of the roots negatively affects both the plant and the pathogen; as the growth of the root is limited, the amount of exudates on the rhizosphere decrease and in consequence the attraction of zoospores, thus the damage they cause, is restricted (Zentmyer 1980). On avocados, stress caused by salinity significantly limits the growth of the roots (Bernstein *et al*. 1996). Considering that root regeneration is a defence mechanisms of avocado against root rot (Kellan and Coffey 1985), it has been argued that, as occurs in citrus, the effect of disease-increase under saline soils could be caused by an elevated susceptibility of the host and/or by an inhibition of root growth and regeneration (Blaker and MacDonald 1986).

Control of Phytophthora root rot

Although Phytophthora root rot of avocados has been widely studied, no definitive control measures have been found. With the pathogen still being the major limiting factor in most world avocado producing areas, many and varied approaches have been developed to reduce the impact of the disease and to allow avocados to survive in the presence of the pathogen. Major control strategies for disease control are summarized below. Reasonable accomplishment of these different procedures could achieve the goals of integrated plant protection; primarily to prevent and, if infection occurs, to coexist with the pathogen, without eradication but limiting the population, improving appropriate environmental conditions for root development and plant growth and heighten production despite the pathogen existence (Coffey 1984).

Diagnosis of Phytophthora cinnamomi

Prevention of the introduction of the pathogen into the orchard is obviously the best control approach. Therefore, the improvement of fast and precise diagnostic procedures is essential to control the introduction of the pathogen into an area. Traditionally, the isolation of the pathogen from roots or soil has been performed by bating or by culture-plating the samples in selective media amended with fungicide and antibiotics (Tsao 1983). Further identification of *P. cinnamomi* has normally been based on morphological and cultural criteria, which require extensive knowledge on oomycetes. Additionally, biochemical probes determining electrophoretic patterns of enzymes and other proteins have been used to differentiate *P. cinnamomi* from other *Phytophthora* species (Clare and Zentmyer 1966; Hall *et al.* 1969; Oudemans and Coffey 1991). Antibodies have also been used in the detection and diagnosis of *P. cinnamomi.* Hardham *et al* (1986) described species-specific monoclonal antibodies against *P. cinnamomi* spore components, which were used latterly in the development of different diagnostic tests used for soil or plant samples (i.e. immunofluorescence assays, ELISAs or a dipstick assay) (Gabor *et al.* 1993; Cahill and Hardham 1994).

Molecular biology techniques based on differences in nucleic acid sequences among species, favoured the detection of *P. cinnamomi* and other *Phytophthora* species. Initially, internal transcriber spacers (ITS) of ribosomal DNA (rDNA) sequences were used to design probes which specifically hybridise with *P. cinnamomi* rDNA amplified by the polymerase chain reaction (PCR) from pure cultures (Lee *et al.* 1993; Lévesque *et al.* 1998) (**Table 1**). Random amplified polymorphic DNA (RAPD-PCR) technique has also been used to generate a species-specific probe for *P. cinnamomi* (Dobrowolsky and O'Brien 1993). Moreover, Judelson and Messenger-Routh (1996) described a species-specific DNA probe for quantitation of *P. cinnamomi* in avocado roots. Coelho *et al.* (1997) developed specific primers derived from the elicitin cinamomin gene for PCR-diagnosis from *P. cinnamomi* pure cultures. ITS region fragments amplified by different primers and further digested by restriction enzymes have also been used for identification of *P. cinnamomi* or *Phytophthora* species (White *et al*. 1990; Ristaino *et al.* 1998; Cooke *et al.* 2000b; Drenth *et al*. 2005). Bailey *et al*. (2002) identified species of *Phytophthora* and *Pythium* by using sequences of the ITS1 region of rDNA as capture probes for PCR ELISA. Kong *et al.* (2003a) further developed the technique of single-strand-conformation polymorphisms to differentiate species of *Phytophthora* based on differences in electrophoretic mobility of single-strand molecules of rDNA. Specific primers for identification of *P. cinnamomi* derived from the *Lpv* putative storage protein genes have also been developed (Kong *et al*. 2003b). Based on specific primers of *Phytophthora* spp. designed by Drenth *et al*. (2005), a novel com-

Table 1 Specific primers and probes used for molecular diagnosis of *Phytophthora cinnamomi.*

Primer and probe	Sequence (5′-3′)	Tm (ºC)	Size (pb)	Target	Author
Probe	CAGTGATAGGGCCCGCCACG	68	-	ITS I	Lee *et al.* 1993
95.422 - Forward primer	GCTCGTGAGTATCCTGTCCG	62	349	Cin-6a	Coelho *et al.* 1997[a]
96.007 - Reverse primer	CTCAGTAAATGGCTAGCCGATAC				
Probe	CGTGGCGGGCCCTATCACTG	68	-	ITS I	Lévesque *et al.* 1998[b]
Probe	ACGGTTGTCTGTTGCGTGGG	64	-	ITS I	Bailey *et al.* 2002
Lpv1 - Forward primer	CTGGCGGCATTGAAGCAAGA	65	412	Lpv	Kong *et al*. 2003[c]
Lpv1 - Reverse primer	CAAGCGCACAGAACGGAGAT				
Lpv2 - Forward primer	ACTGGGTCGACAACGACTGCTTG	60	489	Lpv	Kong e*t al*. 2003[c]
Lpv2 - Reverse primer	GTCCAAACCGACTCTTGCTCGATG				
Lpv3 - Forward primer	GTGCAGACTGTCGATGTG	60	450	Lpv	Kong *et al*. 2003[c]
Lpv3 - Reverse primer	GAACCACAACAGGCACGT				

[a]: PCR program: 3 min at 94°C for initial denaturing, 35 cycles: 1 min at 94°C for denaturing, 1 min at 62°C for anneling, 30 s at 72°C for extension and 7 min at 72°C for final extension.
[b]: Forward version of probe from Lee *et al.* (1993).
[c]: PCR program: 2 min at 96°C for initial denaturing, 39 cycles: 30 s at 94°C for denaturing, 45 s at 60 or 65°C for annealing, 1 min at 72°C for extension and 10 min at 72°C for final extension.

mercial diagnostic kit (Phytophthora-IDENTIKIT^{TM}) have been developed in Australia by C-Qentec Diagnostics Pty Ltd.

Technical management and cultural methods

Production and distribution of clean nursery plants. Sanitary procedures in the nursery should be aimed at preventing the introduction of the pathogen through the soil, tools or water and its dissemination into the nursery areas in order to attain pathogen-free plants. Clean seeds of avocado treated with hot water (30 min at 48-52°C) and clean potting soil treated by the use of fumigants or by steam pasteurisation or sterilization are necessary (Zentmyer 1980). Clean water from deep wells or disinfected with copper sulfate (20 ppm) or chlorine (0.5 ppm) is recommended. Nowadays, the presence of inoculum of important pathogens in water from rivers, canals or reservoirs is a sanitary problem of significant relevance (Hong and Moorman 2005). Other important sanitary procedures are: periodic sampling of roots to detect the presence of *P. cinnamomi* on nursery stock and elimination of infested material, elevation of benches from the ground to prevent contact with soil water after irrigation or rain and protection of nurseries from excessive human, animal or vehicle traffic as they could also introduce the pathogen (Zentmyer 1980).

Site selection and preparation and grove sanitation. Conducive soils for Phytophthora root rot are heavy clay soils, poorly drained with slow subsoil permeability. Soils with high salinity levels are also favourable for root rot development. Good results may be attained by planting trees on mounds to help root development and provide well-drained soils (Goodall *et al.* 1987). On sloped lands, the construction of drainage canals may help to prevent the introduction of *P. cinnamomi* in infested water from contaminated areas after heavy rains. Sanitary procedures should be designed to exclude the pathogen from healthy orchards. The construction of fences to control the traffic of persons, animals or vehicles, the use of disinfested tools and the use of clean water, are necessary in order to prevent the introduction of the pathogen from diseased groves into healthy ones and its dissemination into the orchard (Zentmyer 1980).

Irrigation management. Irrigation water management and soil drainage are measures aimed at reducing water accumulation in the soil and to prevent its surface runoff. The use of tensiometers to maintain adequate soil moisture, considering the local evapotranspiration demands, is strongly recommended (Zenymyer 1980).

Fertilizer nutrients. Healthy and vigorous avocado trees are able to defend themselves more effectively against Phytophthora root rot. Leaf analysis is also important to determine nutrient deficiencies. Ammonium nitrogen fertilizers are less conducive than nitrate fertilizers (Pegg *et al*. 1982). Calcium is an important nutrient that may be useful in the control of avocado root rot. In California, Messenger *et al*. (2000) demonstrated that gypsum amendments to avocado soil (ranging between 1%-5%) reduced sporangial production, sporangial size and zoospore production of *P. cinnamomi*. In orchards, they recommend applications between 1,500 and 3,000 kg ha^{-1} of gypsum under the tree canopies, depending on the tree size.

Chemical control

Before planting, the population of *P. cinnamomi* in the soil can be significantly reduced by soil fumigation (Zentmyer 1980). However, chemical fumigation of infested soils is not recommended because the long-term nature of perennial crops makes the use of preplant fumigants practical for only two-three years. Besides *P. cinnamomi* reinvasion of fumigated soils could become even more efficient where microbial-competitive communities are severely reduced. Moreover, health and environmental negative effects do not support their use. On infested soils, conducive to Phytophhtora root rot, replacement of avocado trees with alternative plants, resistant to the pathogen has been recommended (Zentmyer 1980).

Once established in a plantation, soil pathogens are difficult to eradicate; however, root rots caused by *P. cinnamomi* have been treated with different fungicides to at least reduce the inoculum level. During the 1930s, copper fungicides such as Bordeaux mixture or copper sulphate were recommended (Mehrlich 1932; Oyler and Bewley 1937). Later, numerous soil fungicides were evaluated against the pathogen (Zentmyer 1955a; Zentmyer and Erspamer 1957; Munnecke *et al.* 1974). Among them, fungicides such as fenaminosulf (*p*-Dimethylaminobenzenediazo sodium sulfonate) or ethazol (5-Ethoxy-3-trichloromethyl-1,2,4-thiadiazole) proved the most effective and they have been used against the pathogen on different crops despite their low persistence in the soil and high cost of the need for repeated applications (Milne and Chamberlain 1971; Zentmyer 1973; Frossard and Bourdeaut 1974; Zentmyer 1980). In 1977, two new groups of organic fungicides to control diseases caused by oomycetes were identified; the phenylamides (acylalanines) (Schwinn *et al.* 1977), which includes the metalaxyl and the furalaxyl, and the phosphonates (Bertrand *et al.* 1977), such as fosetyl Al and fosetyl Na. Both chemicals, although with different modes of action, have upward and downward systemic activity, therefore proving effective when applied as soil drench or foliar sprays. Later, at the beginning of the 1980s, different authors reported that phosphorous acid (H_3PO_3) inhibits the mycelial growth of several *Phytophthora* species. *P. cinnamomi* was classified as a high sensitivity species to fosetyl Al and its active metabolite: phosphorous acid (*in vitro* total growth inhibition at 0.42 mM on corn meal agar) (Bompeix and Saindrenan 1984; Fenn and Coffey 1984). *In vitro*, phosphorous acid reduces the sporangia production of *P. cinnamomi* (EC_{50} = 1.8 µg/ml), the zoospore release (EC_{50} = 6 µg/ml), chlamydospores production (EC_{50} = 15-44 µg/ml), and mycelial growth (EC_{50} = 4.1-6.2 µg/ml) (Coffey and Joseph 1985). The elevated solubility of fosetyl Al and metalaxyl permitted applications by trunk injections, which performed satisfactorily in the chemical control of avocado root rot (Darvas *et al.* 1983, 1984; Pegg *et al.* 1985, 1987). Subsequently, salts or esters of phosphorous acid, such as sodium or potassium phosphonates, have been used as fungicides, having both acro- and basipetal movements in plants. Trunk injections of avocados with potassium orthophosphonate, using 20% a.i. solution injected at a rate of 15 ml per metre of tree diameter, also proved efficient for avocado root rot control (Whiley *et al.* 1992). An advantage of trunk injections or foliar sprays when applied against soilborne pathogens is that degradation by soil microorganisms is avoided (Mckenzie and Margot 1982). On the other hand, selection of isolates of *P. cinnamomi* resistant to these phosphorous acid chemical derivates has been reported (Darvas and Becker 1984; Duvenhage and Köhne 1997). The mode of action of phosphorous acid and phosphonates is complex, despite a direct fungistatic effect on the pathogen, an indirect effect by activation of host defence responses has been reported (Bompeix and Saindrenan 1984; Smillie *et al.* 1989; Guest and Grant 1991). More recently, application of soluble potassium silicate (20.7% of silicon dioxide) is under study as an alternative control measure for Phytophthora root rot (Bekker *et al*. 2007).

Whit regard to the use of essential oils in the control of *P. cinnamomi* there are currently very few reports. Whitfield *et al.* (1981) studied the effect of volatile components from the roots of *Acacia pulchella* on *P. cinnamomi* and confirmed that steam-volatile extract restricted mycelial growth, suppressed sporangial production and germination and reduced zoospore germination of the pathogen when grown axenically.

Physical control

One of the most effective physical-methods for soil disin-

festations is artificial heating by water steam (temperatures ranged at 60-100°C). However, important negative biological effects arise such as phytotoxicity or soil vacuum, which implies rapid reinfestation by a low-diversified soil microbiota. Technical limitation is also an issue as it is expensive and difficult to apply (Chen *et al.* 1991). In 1976, Katan *et al.* developed in Israel an environmentally-friendly alternative soil desinfestation technique named soil solarization, which is based on solar heating of soil by polyethylene mulching. It has since been applied in different countries against other soilborne diseases in bare or established groves.

In an avocado grove in Israel, with soil naturally infected by *P. cinnamomi*, Pinkas *et al.* (1984) found that soil solarization (soil covered with transparent polyethylene during six weeks in summer) reduced the percentage of soil infestation from 69% to 3%. In laboratory experiments they found that only 10% of the pathogen propagules survived 4 h of heating at 36°C. In South Africa, Barbercheck and von Bromembsen (1986) demonstrated that soil solarization lasting for 3 weeks eliminated *P. cinnamomi* from 91% of buried artificial inoculum at 10 and 30 cm of depth, whereas with solarization for 6 weeks the treatment completely eradicated the pathogen. Chlamydospores of *P. cinnamomi* were inactivated by exposure to 38 and 41°C for 30 min and to 44°C for 10 min. In California, Juarez-Palacios *et al.* (1991) confirmed inactivation of *P. cinnamomi* in solarized bare soil after two and four weeks at 30 cm and 45 cm depth respectively. *In vitro*, treatments of soils at 45°C for 20 min killed chlamydospores of *P. cinnamomi.* In Spain, López Herrera *et al*. (1996) studied the effect of soil solarization in established orchards affected by root rot. They confirmed a reduction of *P. cinnamomi* on naturally infected avocado roots at a depth of 30-60 cm after 6-8 weeks of solarization during summer. The long-term effect of solarization was also demonstrated as the pathogen could not be detected in avocado rootlets up to 14 months after the treatment. On the Canary Islands, Gallo *et al.* (2007a) evaluated the effectiveness of preplant soil solarization in the further development of root rot. Five years after the treatment, they found that the disease severity of avocado trees planted on preplant-solarized soil was of 2.03 whilst for the untreated control it was of 4.65.

Resistance

The use of plants expressing increased natural resistance against certain pathogens is one of the most efficient methods to control diseases caused by soilborne pathogens. In this context, the approach to the control of Phytophthora root rot of avocado by resistance has involved two different aspects; the search for resistant avocado rootstocks and also the search for suitable resistant plants to use as replacement crops.

The selection of avocado rootstocks resistant to *P. cinnamomi* was initiated during the 1950s in California (Zentmyer 1952). Conventional methods for selection of avocado resistant to *P. cinnamomi* have been based on the immersion of roots in tanks with an inoculated nutrient solution or growing plants in infested soil (Zentmyer and Mircetich 1965). Indirect but faster methods such a callus or stem tissues inoculation has been also developed (Botha *et al.* 1990; van der Merwe *et al.* 1992). Initially, the search for resistant germplasm against *P. cinnamomi* was focused on the area from Mexico to Ecuador with emphasis on primitive types of *P. americana* and on specimens growing in wet areas, if possible in the presence of the pathogen (Zentmyer 1980). Subsequently, seeds and scions of avocado and related species have been collected for their evaluation from diverse regions of the world, mostly in the Americas, but also from old local avocado trees growing elsewhere in an area where most of the trees have died from Phytophthora root rot. As a result of these studies, during the sixties, in tests carried out in California, the 'Duke' cultivar, a Mexican-type avocado expressing moderate degree of resistance (tolerance) was selected, and two 'Duke' seedlings; 'Duke-6' and 'Duke-7', with the best growth in infested soil were selected and propagated by cutting. Currently, these clonal avocado rootstocks are extensively cultivated in many countries. However, novel rootstocks such as 'G6', 'G775' ('Martín Grande'), 'Thomas' or 'Dusa' with higher level of resistance have since been selected and they are alternatives to long-established 'Duke' clones (Gabor and Coffey 1990). Is important to point out that under severe infection conditions, namely poorly drained soils with excess moisture or elevated inoculum density, even these tolerant rootstocks will be damaged. Thus, the search for races and varieties with higher resistance and, in addition, adapted to local conditions and improved agronomic behaviour still continues in different countries. In Chile, a project designed to identify and select national ecotypes with potential tolerance to *P. cinnamomi* plus acclimatisation to salinity conditions and high levels of production is under development (Castro *et al*. 2007). In the Canary Islands (Spain), Gallo *et al*. (2007b) have selected West Indian rootstocks adapted to local conditions and tolerant to *P. cinnamomi,* such as 'Canarias-1' and 'Canarias-2', which perform better than conventional commercial Mexican rootstocks. In South Africa, the local rootstock selection 'Dusa'^{TM} out-performed 'Duke-7' in relation to tree health and yield; additionally new plant material is under evaluation (Kremer-Köhne and Köhne 2007). Likewise, in Australia cloned or seedling plants from rootstocks subjected to selection pressure by *P. cinnamomi* for a long time and grafted to 'Hass' are being compared for their tolerance and yield under different environments with resistant rootstocks developed in other areas (Whiley *et al*. 2007). In California (USA), Douhan *et al.* (2006) have recently incorporated novel technologies using molecular markers assisting the breeding process for the selection of rootstocks tolerant to *P. cinnamomi*.

Nowadays, biotechnological approaches such as somatic embryogenesis offer a complementary technology to traditional breeding programmes. It can allow widening of the genetic base by generating variability *in vitro* (somaclonal variation), through somatic hybridisation or by direct introduction of genes (genetic transformation) (Sánchez-Romero *et al.* 2006).

As previously observed, in areas severely infested by *P. cinnamomi*, the use of suitable resistant plants as replacement crops is recommended. Several species of *Anona*, *Citrus*, *Diospyros* or *Macadamia* among others can be replanted in infested soils, as they are resistant to the pathogen. This feasible approach needs to be adapted to the particular environments and economic requirements of a given area (Zentmyer 1980).

With regard to the basis of resistance in avocado, several aspects were studied. The presence of the chemical borbonol, which has antifungal activity, has been detected in tissues of several *Persea* spp. showing different degrees of resistance to *P. cinnamomi* (Zaki *et al.* 1980). Variations in amino acid composition of root exudates, implicated in chemotaxic attraction of zoospores, have also been associated with different levels of tolerance (Botha and Kotzé 1989). Zoospore attraction, germination and penetration of roots occur in both resistant and susceptible plants, although differences in germination percentages or size lesions have been reported (Ho and Zentmyer 1977b; Aveling and Rijkenberg 1991). A post-penetration host-pathogen interaction seems to play a principal role in these responses ('Duke 7','G 6' and 'Martín Grande' selections) (Aveling and Rijkenberg 1989). Histological studies of moderately resistant and susceptible avocado roots demonstrated that two major anatomical changes; the formation of necrophylactic periderm in the cortex and the isolation of infected phloem bundles by whorls of cells formed by periclinal cell wall division, are designed to separate the pathogen-infected area from the non-infected in tolerant plants (Phillips *et al.* 1987). Phillips *et al*. (1991) studied callus tissues of resistant and susceptible genotypes and concluded that a hypersensitive response is related to defences in avocado. Ad-

ditionally, the presence of suberin-like and lignin-like materials on walls of cortical and stele cells of roots occur on cv. 'Topa Topa' when inoculated with a low virulence isolate. Conversely, when inoculated with a high virulence isolate, these materials are absent and larger necrotic areas are observed (Pozniak and Pinkas 1996).

Suppressive soils and biological control

The biotic interactions that occur between soil microbiota and phytopathogens are one of the most important factors determining the presence, distribution and parasitism of soilborne pathogens. Use of these interrelationships has been attempted over a lengthy period in order to attain a general or specific biocontrol of soilborne diseases, mainly through the use of organic amendments or the addition of biocontrol agents: strategies which could improve the suppressiveness of substrates or soils.

Suppressive soils have long been recognised in relation to other root pathogens. In such soils, the pathogens do not become established. Broadbent and Baker (1974) reported the existence of suppressive soils against *P. cinnamomi* in Australia, where healthy avocado trees coexist with the pathogen. These soils were managed (following the "Ashburner system") to recreate rainforest conditions. These authors indicate that soils suppressive to *P. cinnamomi* tend to have a high content of organic matter, high calcium levels, pH of 5.5 to 5.7, high levels of ammonium and nitrate nitrogen, high biological activity and are well-drained. Moreover, they confirmed that in these soils, *P. cinnamomi* produced fewer sporangia and mycelial growth was weaker than in the opposite conducive soils. The existence of suppressive soils to avocado root rot in South Africa and other areas has been further recognized (Duvenhage *et al.* 1991; Rahimian and Casale 1992).

Although the positive effect of different organic amendments such as alfalfa meal or chicken manure to control *P. cinnamomi* was identified early (Zentmyer 1963), following the description of suppressive soil to *P. cinnamomi*, the control of avocado root rot by application of green manures, animal manures, stable manures or composts, which appears to induce soil suppressiveness, has since been actively examined (Zentmyer 1980). Jacobo *et al.* (1990) confirmed that the incorporation of bovine manure (120 kg/ avocado tree) plus appropriated technical management (chemical fertilization, severe pruning, control of weeds and pests, and individual tree irrigation) reduced *P. cinnamomi* incidence. The use of vermi-compost (a mix of worm-composted sludge and straw) mixed into the top 30 cm of mounds (rate of 20 L of compost per 20 L of soil) protects and increases the survival of avocado trees (Bender *et al.* 1992). Casale *et al*. (1995) evaluated the suitability of urban and agricultural waste products to root health of avocado finding that products which mimicked a forest litter layer (urban yard waste) were the bests for growth and health of avocado and also that they performed favorably in supporting the growth of microbial biocontrol agents (*Trichoderma viride*, *Gliocladium virens* and *Pseudomonas fluorescens*). Costa *et al.* (1996) correlated the organic matter levels with the ability of mulches to trap zoospores and reduce the disease incidence identifying that yardwaste mulches enhanced aborted sporangia production. Aryantha *et al.* (2000) demonstrated that fresh chicken manure or chicken manure composted for 5 weeks and incorporated to potting mixes (25% v/v) reduced the survival of *P. cinnamomi* and the development of root rot symptoms on *Lupinus albus* seedlings. Moreover, these authors associated the survival of plants with increased organic matter content, total biological activity and populations of actinomycetes, fluorescent pseudomonas and fungi specifically with the stimulation of endospore-forming bacteria. Downer *et al.* (2001a, 2001b), proposed that the production of cellulase and laminarinase by litter decays saprophytic fungi and is a mechanism by which mulches soils, thus simulating the rain forest conditions, reduces Phytophthora root rot. They concluded that both enzymes, which have different effects on the development and production of the different reproduction and survival structures of the pathogen, might have a significant role in the reduction of *P. cinnamomi.* In mulched soils, the reduction of abiotic disease-predisponsing factors such as fluctuations in soil temperature, salinity or water logging, may also operate regulating the disease (Downer *et al.* 2002).

Moreover, the existence of suppressive soils with a natural capacity to control *P. cinnamomi*, implied that numerous tests of microorganisms to try to prove their antagonistic behaviour should be performed. In natural soils it is known that lysis of the mycelium is associated with intense bacterial colonization of hyphae and the breakdown of cytoplasmic content, and that lysis of mycelium is associated with the formation of zoosporangia and chlamydospores (Malajczuk 1983). On the other hand, earlier studies recognized that the production of either sporangia or oospores of *P. cinnamomi* is stimulated by metabolites from certain bacteria or fungi. Therefore, suppressive soils, where the pathogen failed to produce sporangia (Broadbent and Baker 1974), could be soils in which sporangia-stimulating-bacteria are themselves suppressed (Malajczuk 1983). Furthermore, soil-bacteria associated with the pathogen hyphae may play an important role in biological control. Invariably, microbial populations of suppressive and conducive soils and of the rhizosphere of plants growing in them are quantitatively and qualitatively different. Thus, specific antagonistic isolates of bacteria and fungal species have been selected from suppressive soils or avocado rhizosphere following different strategies and their biocontrol potential towards *P. cinnamomi* examined. *Myrothecium roridum* (strain TW), selected from among 36 fungi and 110 bacteria from the rhizosphere of avocado roots growing in suppressive soils, was proved to be an active antagonist and to suppress *P. cinnamomi* in greenhouses potting mixes and naturally infested soils (Gees and Coffey 1989). Finlay and McCraken (1991) studied a wide range of microorganisms (*Streptomyces* spp., Basidiomycetes, *Mortierella* spp., *Trichoderma* spp. and *Epicoccum* spp.) shown to have antagonistic properties against *P. cinnamomi* in paired cultures on agar media. Stirling *et al*. (1992) isolated three fluorescent pseudomonads, nine actinomycetes and a *Serratia* sp. from roots of avocado trees growing in a soil suppressive to *P. cinnamomi* with *in vitro* antagonistic activity against the pathogen. Duvenhage and Kotzé (1993) identified antagonistic fungal isolates of *Aspergillus candidus*, *Paecilomyces lilacinus* and *T. hamatum* and antagonistic bacterial isolates of *Bacillus azotoformans* and *B. megaterium*, which reduced the development of the disease on avocado seedlings and even under field conditions (Duvenhage and Köhne 1997). Domínguez-Correa *et al*. (1999) selected from forest soils, strains of *P. putida* and *B. polymixa* antagonistic to *P. cinnamomi*. Yin *et al*. (2004) developed a new strategy to select bacteria which fills a niche similar to that of the pathogen, hypothesizing that they could suppress the disease through nutrient competition and antibiosis. Martín-Sánchez *et al*. (2007) selected strains of *P. chlororaphis* from avocado roots with biocontrol against both *P. cinnamomi* and the aggressive root pathogen *R. necatrix* on avocado seedlings. Development of the application of biocontrol agents to substrates, mainly in nursery industries, has been achieved. Steddom and Menge (2001) validated a commercial field fermentor (called Bioject, Eco Soil Systems, CA) for producing and delivering bacterial biocontrol agents (*P. putida* 06909-rif/nal at different concentrations) for commercial-scale field applications.

Similary, arbuscular mycorrhizal (AM) fungi, mainly species of *Glomus*, have been confirmed to have a positive effect on survival, growth and nutrition of avocado seedling plants and on micropropagated plants. Menge *et al.* (1980) confirmed an increased growth of avocado 'Topa Topa' seedling of 49-254% when incolulated with the AM fungus *G. fasciculatus*. Azcón-Aguilar *et al.* (1992) and Vidal *et al.* (1992) demostrated that *Glomus* spp. (1 g of clean mycorrhizal-onion-roots per plant) improved the sur-

vival and development of micropropagated avocado plants when applied at the beginning or after the acclimatization. However, the bioprotective effect of AM fungi on avocado plants against *P. cinnamomi* has not been totally elucidated. In early experiments, Mataré and Hatting (1978) concluded that *G. fasciculatus* seems unlikely to have any important effect on avocado seedling infection by *P. cinnamomi* and disease development. Meanwhile, Davis *et al.* (1978) found that avocado seedlings were more severely affected by *P. cinnamomi* than were nonmycorrhizal plants, concluding that the phosphorous nutritional status of the plant may have an important role in the effect of AM-avocado-*P. cinnamomi* interactions. To respond to this significant question more research is required since the level of protection is highly plant species-AM fungi isolate specific. Over the coming years, with the improvement of monoxenic inoculum production technologies, which will provide axenic cultures of AM fungi for more precise studies, further light will be shed on this issue (Pérez-Jiménez *et al.* 2006).

OTHER SIGNIFICANT DISEASES

Anthracnose

Anthracnose is a very common postharvest disease. It is endemic in most producing areas and is the most important fruit disease in high-rainfall growing areas (Darvas and Kotzé 1981; Fitzel 1987; Fucikovsky and Luna 1987; Hartill 1991). The mature avocado fruit is rotted; consequently, very important losses occur during commercialisation. In a market survey in South Africa, Sanders *et al.* (2000) recorded incidences on overripe avocados up to 80%. In other studies, losses of 71% on unripe fruits (Fitzel 1987) or 36% on mature fruits have been recorded (cited in Korsten *et al*. 1991). Therefore, in some countries, the disease requires continuous control measures.

Symptoms

On harvested fruits, initial symptoms are small light-brown spots on the surface. As the fruit ripens and the fungus develops, the lesions become sunken and darker, rotting the flesh with the spots developing and converging as large necrotic areas. Simultaneously, under high humidity conditions, salmon-coloured spore masses are produced abundantly on the surface of the lesions (**Fig. 3**). Preharvest anthracnose symptoms can develop associated with skin injury or high inoculum concentrations. Symptoms of the disease can also be observed on different organs of avocado trees. Under very humid conditions symptoms may be present on leaves as necrotic brown spots and defoliation can occur. On shoots, brown or purple lesions with white exudates can be observed and, on branches, wilting is followed by dieback. Infected inflorescences present dark lesions and in some cases flowers fall and young fruits abort (Darvas and Kotzé 1981; Fitzel 1987; Fucikovsky and Luna 1987; Hartill 1991). *C. gloeosporioides* may also develop on avocado fruit as a different preharvest disease named pepper spot or speckle (Willingham *et al.* 2000).

Causal agents

The ascomycetous fungus *Glomerrella cingulata* (anamorph *Colletotrichum gloeosporioides*) is the principal pathogen associated with anthracnosis in all avocado producing countries. Nevertheless, the species *Glomerrella acutata* (anamorph *Colletotrichum acutatum*) is also pathogenic to avocados and has been described in different areas (Hartill 1991; Peres *et al.* 2002; Guillén-Andrade *et al.* 2007). *C. gloeosporioides* is phenotypically more variable and has a wider range of hosts than *C. acutatum*. Colony colours of *C. gloeosporioides* range from pale grey, salmon or dark grey. The fungus produces acervuli which erupt through the epidermis of fruit, where conidia develop. Conidia are variable in shape: cylindrical and straight with a tapered base and obtuse or with pointed ends (Sanders and Korsten 2003). *C. acutatum* produce smaller conidia with pointed ends. The sexual stage of both fungi produces perithecia with ascopspores. Both species may also be molecularly identified using specific primers; *Cg*Int/ITS4 for *C. gloeosporioides* and *Ca*Int2/ITS4 for *C. acutatum* and (Mills *et al*. 1992; Sreenivasaprasad *et al*. 1996).

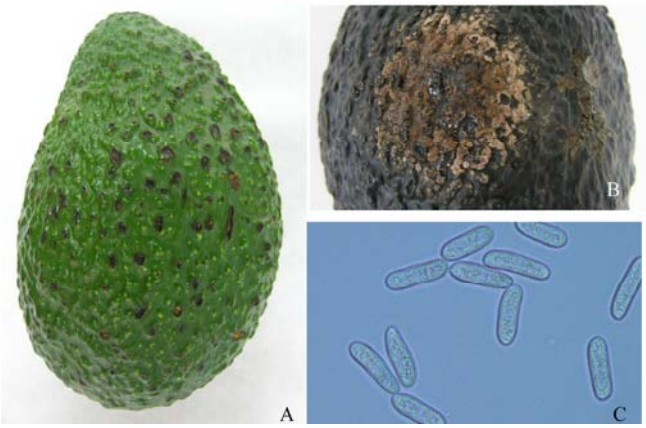

Fig. 3 Initial anthracnose symptoms on unripe avocado fruit cv. 'Hass' (**A**). Mature fruit cv. 'Hass' with advanced external symptoms of anthracnose, typical salmon-coloured spores developed on the surface (**B**). Conidia of *Colletotrichum gloeosporioides* (**C**) (×1000).

Epidemiology

In general, high humidity (above 80%) and warm temperatures (between 18 and 26°C) promote infections, therefore, in areas with dry summers the disease in not significant. Acervuli and/or ascomata occur normally on senescent tissues such as fruits, leaves or twigs, although teleomorphs seem to play a minor role in the epidemiology of the disease. The fungus spreads from diseased organs, from the soil or tree, to healthy organs and sporulates on them (Fitzel 1987). Heavy rains and high winds favour conidial dispersal, although on other hosts, dew has been seen to be enough for conidia-spreading. On fruits, infection can take place at any phase of the developmental stage; conidium germinate forming a germ tube and a terminal appressorium from which an infection peg emerges and penetrates the outer wax layer and cuticle of the fruit skin (Binyamini and Schiffmann-Nadel 1972). Nevertheless, as described by Prusky *et al*. (1983), the presence on unripe fruit of antifungal compounds (i.e. epicatechin) limit the growth of the infection peg, which remains in latency until fruit ripening. During ripening, levels of these compounds decrease by enzymatic action (i.e. laccase) (Guestsky *et al.* 2007). Then, latent infections become re-activated and the hyphae invade the flesh until most of the fruit is rotted. In advanced stages acervuli are produced beneath the fruit surface. When the cuticle and epidermal cells are ruptured conidia are released and dispersed by water.

Control

Cultural practices, preharvest and postharvest chemical or biological treatments in combination with the best possible storage conditions can reduce the incidence of anthracnose on avocado trees, and consequently on mature fruits. As occurs in most postharvest diseases, inoculum sources such as mummified fruits, dead twigs and branches of avocado or other hosts plants must be removed from the orchard. Aerating the trees through selective pruning of dense trees can reduce the humidity level (Fitzell 1987). In relation to plant resistance, cultivars 'Fuerte', 'Rincon' and 'Wurtz' are more susceptible than 'Hass'. In addition, severity and incidence of anthracnose on 'Hass' fruit are significantly reduced when 'Hass' is grafted to Guatemalan race rootstocks

instead of to the more susceptible Mexican race (Whiley *et al*. 2007). These improvements in postharvest diseases are associated with lower concentrations of nitrogen and potassium, higher calcium and magnesium, lower ratios of nitrogen : calcium and higher ratios of calcium + magnesium : potassium in 'Hass' on 'Velvick' compared with 'Hass' on 'Duke 6' in fruit and leaves (Willingham *et al*. 2006). Once collected, storage temperatures ranging from 5 to 18°C reduce disease development, whilts temperatures above 24°C favour its progress.

Preharvest chemical treatments with different copper fungicides such as copper oxychloride (0.25%) and copper hydroxide (0.15%) alone or in combination with other fungicides such as captafol (0.16%) or benomyl (0.025%) are effective when floral buds begin to swell and throughout the fruit development period (intervals of 14-28 days) (Peterson and Inch, 1980; Darvas and Kotzé, 1987). Although with increasing restrictions, postharvest fungicidal treatments with prochloraz (0.5%) have been shown to be effective against *Colletotrichum* spp. (Darvas and Kotzé, 1987). Biological control of anthracnose has been extensively studied as an alternative to chemical control. In South Africa, Korsten *et al.* (1991) evaluated the strain of *B. subtilis* A6, isolated from avocado phylloplane, against the most important postharvest diseases (anthracnose, fruit rot and stem-end rot) and confirmed that antagonistic water dip treatments (suspensions of *B. subtilis* at 1 x 10^7 or 2.1 x 10^7 cells ml^{-1}) of avocado fruits was as least as effective as prochloraz in controlling these diseases. Further evaluation of the antagonist, marketed as a wettable powder (Avogreen®, at a concentration of 10^9 cells g^{-1} product), on a commercial scale (100 g product $1l^{-1}$ of commercial wax) gave erratic results, possibly due to product formulation (Korsten *et al*. 1998). In Australia, Stirling *et al.* (1995) selected *Bacillus* spp. and yeasts from avocado phyllosphere that reduced lesion development and size on detached avocado when applied (at 10^8 to 10^9 spores ml^{-1}) on artificially *C. gloeosporioides*-inoculated avocado fruits. In Mexico, Vidales-Fernández *et al.* (2007) have demonstrated the potential of *Thichoderma harzianum* (Amicus-L®, 10 monthly treatments at 0.1-0.4%) to control the disease.

Armillaria root rot

Armillaria root rot was first reported by Smoyer in California in 1941. Since then the disease has occasionally been reported in other countries (Darley and Zentmyer 1957; Ploetz *et al.* 1994). The dispersal of the pathogen occurs mainly throughout the soil and by root contact. This disease may be caused by different *Armillaria* species, among them, *A. mellea*, the oak root fungus, seems to be the most distinguished species.

Symptoms

Armillaria infects large roots and the crown of avocado. Above-ground symptoms are noticeable when the fungus has colonised the majority of the root system. Two different symptoms may be observed; in some cases, the vigour of the tree can deteriorate gradually exhibiting chlorotic leaves and slow growth, in others the tree can wilt and die suddenly with the leaves still attached to the tree. Large mycelial plaques white-cream in colour are produced under the rotted bark, and once established in the crown, they can progress upward to the base of the tree (Zentmyer *et al.* 1965). Along the root surface dark rhizomorphs or mycelial strands may be observed with the naked eye. After rainfall the fungus produces typical honey-coloured clusters of basidiocarps under infected trees, hence *A. mellea* is also known as the "honey fungus".

Causal agents

Armillaria species are difficult to differentiate morphologically. The basidiomycete genus *Armillaria* includes more than 40 species. Among them, *A. mellea* is one of the best-known species with a worldwide distribution and a wide range of hosts, mainly in forest ecosystems. *A. mellea* is considered to be a complex of several reproductively isolated groups, equivalent to "biological species" (Anderson and Ullrich 1979). To differentiate species mating tests using known species have frequently been used. *Armillaria* species are isolated from infected tissues on malt agar (20 g l^{-1}) amended with thiabendazole lactate (250 µg l^{-1}), penicillin (100 µg l^{-1}), and streptomycin (100 µg l^{-1}), and incubated in the dark (Guillaumin *et al.* 1982). On plates, *A. mellea* grows slowly and has a red-brownish colour. Rhizomorphs are formed *in vitro*, growing and branching into the medium (**Fig. 4**). Another species described on avocado in Florida is *A. socialis* (Ploetz *et al.* 1994).

Fig. 4 Colony of *Armillaria* sp. growing on PDA. Typical rhizomophs are abundantly formed into the medium.

Epidemiology

Armillaria has a high saprophytic competitive behaviour, thus it can survive on stumps or dead roots buried in the soil for many years. From debris, the fungus grows outward by the formation of rhizomorphs or mycelial strands infecting healthy avocado roots. Contact between the roots of colonized trees and those of healthy trees is the main mechanism for dispersal of the disease, thus diseased patches within the orchard may be present. Basiospores are not considered to infect avocado (Darley and Zentmyer 1957).

Control

Armillaria root rot control has been developed mainly for forest trees and citrus (Munnecke *et al.* 1976; Williams *et al*. 1986). It should be directed toward limiting the dispersal of the disease, providing a good growing environment and proper cultural and sanitation practices. *Armillaria* fungus is very sensitive to drying, thus excavating soil around the trunk to temporarily air-dry the root crown can be effective on avocado. Providing good drainage and avoiding excess irrigation are important. These fungi may remain alive for many years on organic debris, therefore it is crucial to remove dead trees and as many root pieces from the soil as possible and to consider replanting only with crops not susceptible to *Armillaria* (Ploetz *et al.* 1994). Soil fumigation with different chemicals is only partially effective and does not completely eradicate *Armillaria* from the soil, although the pathogen may be weakened and then attacked by other soil mycoparasitic inhabiting fungi such as *Trichoderma* spp. (Ohr *et al*. 1973; Munnecke *et al*. 1981). Moreover, fumigation is expensive and potentially hazardous.

Branch canker and dieback caused by *Botryosphaeria* spp.

Branch canker and dieback are described together in this section in an attempt to provide a more straightforward exposure, as they are usually caused by the same group of pathogens. The original name of the disease "Dothiorella

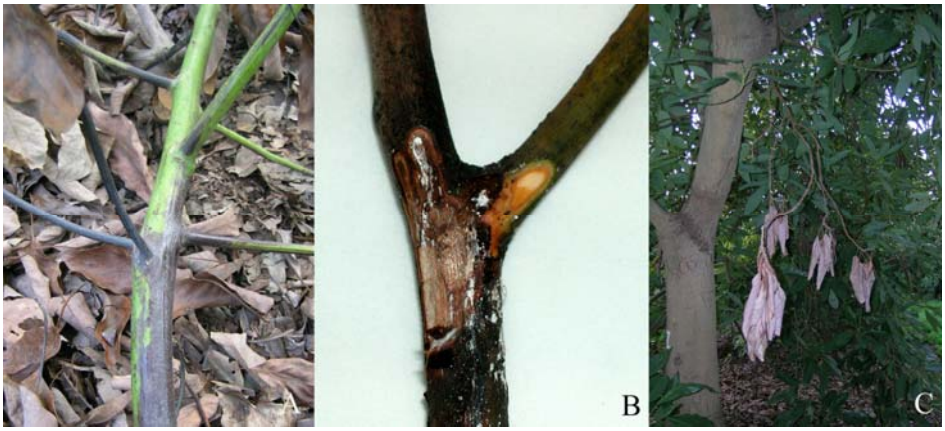

Fig. 5 Symptoms related to the isolation of *Neofusicoccum parvun* from avocado trees in Andalusia (Spain). Black necrotic areas on a young avocado branch (**A**). Advanced stage of the infection with typical white exudates on lesions and necrotic areas occurring under the bark (**B**). Dieback of avocado tree caused by the pathogen (**C**).

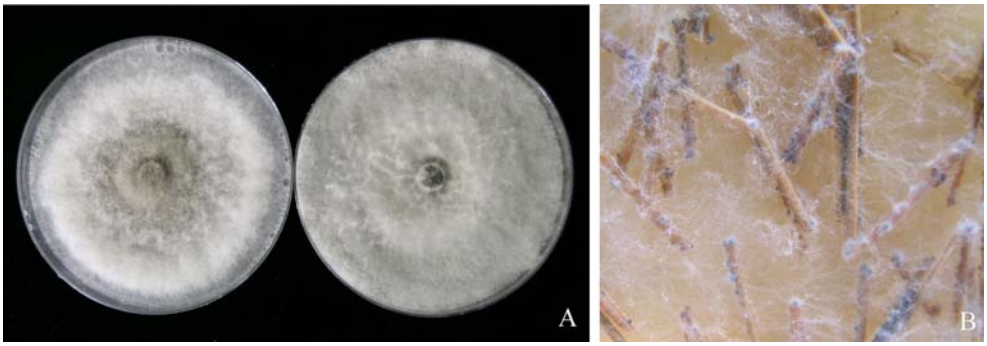

Fig. 6 Colonies of *Neofusicoccum parvum* growing on PDA (**A**). Mycelia and pycnidia formed on water agar with sterilized pine needles as substratum (**B**).

canker" assigned to these symptoms is avoided according to recent taxonomic studies (Crous *et al.* 2006). Actually, several *Botryosphaeria* species, or their associated anamorphs, have been described causing cankers on twigs, branches or trunks and dieback of avocado (Rondón and Guevara 1984; Zea-Bonilla *et al*. 2007a).

Symptoms

On infected branches, black or dark-brown areas in the proximity of the normally greenish, healthy surfaces may be observed. When the disease progresses, the surface of symptomatic areas may be lightly sunken and with a dry appearance. In more advanced stages, white powder which exudes from the bark and a cracking and shedding of the outer bark are detected. When the bark of the cankered area is cut into, it is found to be brown in colour. If the necrosis encircles the branch a dieback occurs and leaves quickly turn brown but remain attached (**Fig. 5**). The normal development of infected trees is affected. In unusually severe cases the tree may be killed (Zentmyer *et al.* 1965; Rondón and Guevara 1984; Zea-Bonilla *et al*. 2007a).

Causal agents

Botryosphaeria is a species-rich genus in which at least 18 anamorph genera have been associated, the most common being *Botryodiplodia* (*nomen dubium*), *Diplodia*, *Dothiorella* (now it is accommodated in *Diplodia*), *Fusicoccum* and *Lasiodiplodia*. The genus has a cosmopolitan distribution and a large morphological and evolutionary complexity. Indeed, within the *Botryosphaeriaceae*, recent molecular and morphological studies have led to new taxonomic re-evaluations and to the descriptions of new taxa within this family (Crous *et al.* 2006). Therefore, there is some confusion between previously described species as causal agents of branch cankers and dieback and the new names of the species related with these symptoms.

Branch canker is most commonly caused by *B. dothidea* (anamoph: *F. aesculi*, previously regarded as *Dothiorella gregaria*), the same fungus that causes fruit rot and stem-end rot as described later. Other *Botryosphaeria*-like species that have also been related to the disease are the anamorphs genera: *Neofusicoccum parvum* (syn. *F. parvum*), *L. theobromae* (previously described on avocado as *Botryodiplodia theobromae*) and *N. ribis* (syn. *F. ribis* and previously named as *D. aromatica*) (Zentmyer *et al*. 1965; Rondón and Guevara 1984; Crous *et al.* 2006; Zea-Bonilla *et al.* 2007a). The identification of *Botryosphaeria* species and their associated anamorphs is based mainly on the morphology and size of the conidia of the anamorphs and on the analyses of the ITS and 5.8 rDNA sequences (Jacobs and Rehner 1998). On potato dextrose agar (PDA), these fungi produced fast-growing white, appressed mycelium that turned dull grey as the colony aged. Abundant pycnidia and conidia develop when isolates are cultured on 2% water agar with sterilized pine needles as substratum at 25°C under near-UV light for 2 weeks (**Fig. 6**).

Epidemiology

Brotryosphaeria species are described as endophytes or more properly as phellophytes according to Hatill and Everett (2002). Symptoms of the infection are evident when the tree is stressed by biotic factors such as other disease or insect attack, and by drought, flooding or nutritional deficiencies. Ascospores and conidia are produced on dead bark, twigs, cankers, senescent fruits and dead leaves. They are disseminated by wind or rain infecting wounds caused by pruning, draughts, wind or frost. Warm temperatures and moist conditions are conducive to the disease (Zentmyer *et al.* 1965; Rondón and Guevara 1984).

Control

As observed on other hosts, pruning activities could efficiently distribute *Botrysophaeria* species (Phillips 2002; Zea-Bonilla *et al.* 2007a). The disinfestations of tools and removal of vegetal debris is strongly recommended. They should not be left close to the tree and care must be taken to ensure that they are eliminated, as they may function as an inoculum source. Symptomatic twigs, branches and old fruits should be removed during dry periods. Mexican varieties have been reported to be much more resistant to the disease than Guatemalan ones; hence they should be used as rootstocks (Zentmyer *et al.* 1965; Rondón and Guevara 1984).

In the case of severe and recurring infection it is useful to spray the infected area with copper fungicides several times during the rainy season. If lesions are abundant, scraping the outer damaged bark will encourage regeneration of vigorous bark. Correction of stress factors will reduce the disease.

Cercospora spot

Cercospora spot is also known as Pseudocercospora spot, blotch or black spot. In South Africa is the most important preharvest disease of avocado and can cause losses up to 69% in untreated orchards (Darvas and Kotzé 1987). The disease is also important in Florida, several areas in Mexico, and Australia, since warm and humid climates are conducive for the disease (Darvas and Kotzé 1979; Ploetz *et al*. 1994; Téliz 2000). Lesions caused by the fungus can occur on leaves, stems or fruits.

Symptoms

The fungus causes small (3-6 mm in diam.) angular spots on leaf margins, initially of a brown colour and later purple. Many of the spots are surrounded by yellow haloes. The specks may enlarge and coalesce, affecting most or whole leaves, which become deformed. The infection may progress along the stem, and young trees may defoliate. Similar small-scattered slightly sunken flecks appear on fruit, which may crack and permit the entry of other pathogens (**Fig. 7**). Sometime the fungus can invade the flesh. Under humid conditions greyish mycelium and fungal spores appear on the surface of the spots (Darvas and Kotzé 1979; Ploetz *et al.* 1994).

Causal agent

The disease is caused by the ascomyceteous fungus *Pseudocercospora purpurea* (syn. *Cercospora purpurea*, teleomorph *Mycosphaerella*) (Deighton 1979). Under high humidity conditions, infected leaves or fruits develop conidiophores on an irregular dark stromata (15-125 µm in diameter). Conidia are obclavated-shaped to cylindrical, with a truncate base, pale olivaceus (2.0-4.5 × 20.0-200.0 µm) and multiseptate. The fungus may be isolated from fresh symptomatic tissues on PDA. The colony is leathery, with tufts of conidiomata on it, initially grey becoming brown with age.

Epidemiology

Infection mainly occurs through conidia, which develop on infected organs under high humidity and warm conditions. Subsequently, water, wind or insects spread them to the infection points. Penetration can be direct or through wounds. After penetration the pathogen remains latent for about three months. Small fruits and mature fruits are immune, whereas intermediate size fruits are susceptible (Ploetz *et al.* 1994).

Control

As a cultural or preventive practice it is important to remove the remains of pruning from the orchard because the fungus may have developed on these. Among cultivars 'Fuerte' and 'Ryan' are more susceptible to the disease than 'Hass' and 'Edranol' (Darvas and Kotzé 1987). In South Africa, where Cercospora spot is considered as the most problematic pre-harvest disease of avocado (Darvas and Kotzé 1979), chemical control has been widely developed and it is currently recommended that Cercospora spot be controlled, as a component of an integrated disease control programme, by monthly applications from October to January of copper oxychloride (0.2-0.3%) alone or in combination with systemic fungicides such as azoxystrobin (0.03%) (Willis and Mavuso 2007). Biocontrol with *Bacillus subtilis* has been achieved by Korsten *et al.* (1992). They demonstrated that the severity of Cercospora spot is reduced when avocado trees are treated with pre harvest sprays of *B. subtillis* (isolate B246, at a concentration of 10^7 cells ml^{-1}) alone or in combination with different fungicides.

Fig. 7 Symptoms of Cercospora spot on fruit and fruit stalk. Brown coloured lesions are surrounded by yellow haloes (courtesy of Anita Willis, South Africa).

Fruit rot caused by *Botryosphaeria* spp.

The disease is also known as Dothiorella fuit rot (Zentmyer *et al.* 1965), although several species of the genus *Botryosphaeria* or its associated anamorphs may cause rot of avocado fruit. The disease was reported to be notable in California, mainly in moist coastal areas, and especially on cv. 'Fuerte' (Zentmyer 1955b). Symptoms of the disease on fruits are indistinguishable from those caused by anthracnose. Therefore, early reports referred to the disease as the Dothiorella/Colletotrichum fruit rot complex (Darvas and Kotzé 1987).

Symptoms

The symptoms only develop on softening fruit after harvest, thus there is no method to detect fruit which will go on to develop this rot. On the green fruit surface small reddish-brown irregular spots may be observed. As the fruit ages, the spots gradually enlarge, sink and turn black and the lesions extend into the pulp and an unpleasant odour develops. These symptoms are similar to those of anthracnose, however in this case the rot is more superficial and sporulation does not occur. Typical grey mycelium of these species may be observed on the fruit surface (Darvas and Kotzé 1987).

Causal agents

The disease is caused by several species of fungi. In general, the same species of the genus *Botryosphaeria* or its associated anamorphs which induce cankers and/or dieback on avocados, such as *B. dothidea*, *Neofusicoccum parvum* or *N. ribis*, have been related with fruit rot of avocados. Most relevant characteristics of these species have been previously described in this review (Branch canker and dieback caused by *Botryosphaeria* spp.).

Epidemiology

As previously noted, conidia and ascospores are abundantly produced on fallen fruits, branches or dead bark. They are normally disseminated by wind and rain, infecting the fruit through wounds and lenticels although direct penetration has also been described (Darvas 1982). After infection the fungus remains dormant and rot does not develop until fruits begin to soften.

Control

To reduce the disease, it is important to remove dead wood or dead leaf tissues from trees in order to reduce inoculum sources (Zentmyer *et al*. 1965). Pruning should be done during dry periods. Trees stressed by rot diseases, drought or nutrient deficiency are more susceptible than healthy trees. It is important to avoid saline conditions because the fungus will survive on dead portions of leaves caused by salinity stress. Preharvest sprays of the trees with copper fungicides (Zentmyer *et al*. 1965) or benomyl (0.0025%) plus captafol (0.08%) gives some control (Darvas 1982; Darvas and Kotzé 1987). Postharvest chemical treatments

with wax alone reduce the occurrence of the pathogen alone or associated with *Colletotrichum* spp. (Darvas and Kotzé 1981). Biological control by dip or wax treatments with *Bacillus subtilis* significantly reduce the disease (Korsten *et al*. 1991).

Phytophthora canker

Several *Phytophthora* species can cause lesions or cankers in the lower trunk of avocados. *P. cinnamomi*, as observed in the first section, was reported early causing trunk cankers under high soil humidity conditions (Zentmyer 1955b). *P. citricola* can cause large cankers in the lower trunk, and the disease it causes is also known as Phytophthora crown canker (Zentmyer *et al.* 1974). It is a significant problem in California (Oudemans and Coffey 1987; Coffey *et al.* 1988). *P. heveae* have also been isolated from cankers or trunks of young trees in Guatemala (Zentmyer *et al.* 1976b). Other species such as *P. palmivora* and *P. boehmeriae* have occasionally been reported causing cankers in Honduras and Mexico respectively (cited in Zentmyer and Jefferson 1973; Téliz 2000).

Symptoms

Phytophthora cankers originate at or below the soil level and may extend 2.0-3.0 m up to the trunk. They have a characteristic sour or fermented odour. The bark manifests discoloration and from the affected area a brownish-red sap, which becomes white when dry, is exuded (**Fig. 8**). *Phytophthora* colonizes phloem and cambium tissues thus the inner bark and outer layer of wood is brown instead of white or cream coloured. Affected trees gradually lose vigour and decline at the top, resembling symptoms exhibited by trees affected by Phytophthora root rot. When the canker encircles the trunk the tree dies suddenly with dry attached leaves (Oudemans and Coffey 1987; Coffey *et al.* 1988).

Causal agents

Amongst the most significant *Phytophthora* spp. causing avocado cankers, *P. citricola* and *P. heveae* are described below; *P. cinnamomi* has been described in the first section of this review. *P. citricola* is an important root pathogen of fruit and forest trees and ornamental plants. Indeed, on avocado *P. citricola* have also been described as causing fruit rot (Koike *et al.* 1987). On PDA, colonies of *P. citricola* have white-dense aerial hyphae with a stellate pattern. Sporangia are noncaducous, semi-papillate, obovoid with 30 to 75 µm long × 21 to 44 µm wide (average 47 × 34 µm). *P. citricola* is homothallic. Paragynous antheridia, spherical oogonia of 25 (18 × 35) µm diameter and plerotic oospores of 22 (16 × 30) µm diameter of *P. citricola* are produced on V8 agar (**Fig. 9**) (Waterhouse and Waterson 1966b; http://www.phytophthoradb.org). *P. heveae* have caducous and papillate sporangia, ovoid, ellipsoid or obpyriform, sometimes irregular, 27 to 66 µm long × 20 to 49 µm wide (average 45 × 29.6 µm). Chlamydospores are not produced. *P. heveae* is homothallic. Amphigynous antheridia and spherical oogonia are of 17 to 32 µm in diameter (average 22.3 µm). Oospores are produced on agar medium, aplerotic, 15 to 26.8 µm in diameter (average 21.5 µm) (Stamps 1978; http://www.phytophthoradb.org).

Fig. 8 Cankers on lower trunk of avocado trees associated with the isolation of *Phytophthora* spp. Brownish-red exudates are formed on the affected area (**A**). Necrotic lesions are present under the bark (**B**).

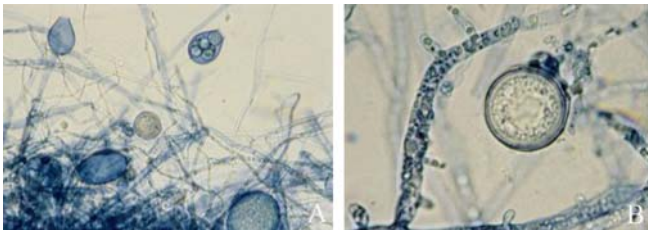

Fig. 9 Morphology of *Phytophthora citricola.* Sporangia developed on liquid medium: empty and zoospore-filled sporangia may be observed (×400) (**A**). Developing oospore on solid medium (×1000) (**B**).

Epidemiology

The epidemiology of *P. citricola* causing avocado cankers has been thoroughly described in California. El-Hamalawi and Menge (1994a) confirmed that the pathogen is unable to penetrate the plant and that the infection always occurs through wounds caused mainly by sucker removal, cultivation practices or animals. El-Hamalawi *et al*. (1995) also confirmed that when infection is localized in the root system it does not progress to the trunk, however abundant zoospores can be produced from roots, especially under saturated soil, infecting wounds of the trunk and developing canker. Continuous wetness of the trunk with water from the sprinklers favours the growth of cankers. Oospores and mycelium are present in exudates hence insects, birds or water, may spread these propagules to other wounded trees. Inoculum from soil may also infect fruits through water splash. Avocado trees are more susceptible to infection of the pathogen when they are stressed by low temperature, salinity, Phytophthora root rot or water stress. The physiology of the tree also affects canker development, being more active during nutrient storage in the bark or after pruning (El-Hamalawi and Menge 1994a).

Control

To prevent Phytophthora canker, it is important to avoid wounding the trunks and not allowing the lower trunk to be wet for long periods. To attain this, mulches must be placed at some distance from the trunk. Also, irrigation water from the sprinkler system splashing soil or water against the trunk must be avoided. Scraping the surface of healed over cankers may result in the reactivation and spread of the canker. When cankers are detected at an early stage they can be controlled by cutting the associated infected bark and painting the treated area with a fungicide such as Bordeaux mixture (copper sulphate and hydrated lime), fosetyl-Al or potassium phosphonate (Coffey and Joseph 1985; Ohr 1990; El-Hamalawi and Menge 1994b). El-Hamalawi *et al*. (1994) characterised the response of nineteen rootstocks selections to Phytophthora canker caused by *P. citricola* and demonstrated that the rootstocks; 'Borchard', 'UC2002', 'UC2003', 'Dusa', 'D9', 'Duke 6', 'UC2011', 'G22', 'Evstro', 'Aguacate mico', 'UC2001', and 'G1033' showed the highest relative resistance (74.5-84.5%) to *P. citricola*.

Pythium root rot

Species of *Pythium* are distributed throughout the world and are important plant and animal pathogens (Hendix and Campbell 1973; van der Plaats-Niterink 1981). Early studies of characterization of avocado-associated fungi and oomycetes referred to the regular presence of *Pythium* spp. on avocado roots and soils (Wager 1942; Harvey 1945). However, their parasitic behavior toward avocado was not fully investigated and they remained considered as weak

pathogens or saprobes. Nevertheless, there is more recent literature referring to *Pythium* spp. as avocado root pathogens (Darvas 1979; Solel and Pinkas 1984; Zea-Bonilla *et al.* 2007b).

Symptoms

Pythium spp. causes necrosis of feeder roots. In nursery plants *Pythium* spp. have repeatedly been reported (Solel and Pinkas 1984). In established orchards, aerial symptoms such as wilting, chlorosis, sparse leaf production and microphylly have recently been related with the isolation of the pathogen from the roots in Andalusia (Zea-Bonilla *et al.* 2007b). Symptoms of Pythium root rot are difficult to distinguish from those of Phytophthora root rot as they both cause necrosis on feeder roots.

Causal agents

Different *Pythium* species such as *P. derbayanun*, *P. spleendens* and *P. ultimum* in South Africa (Darvas 1979), *P. proliferum* in Israel (Solel and Pinkas 1984) and *P. vexans* (Zea-Bonilla *et al.* 2007b) have been described as avocado root pathogens although exhibiting a great variation in aggressiveness. Morphology and measurements of hyphae, asexual and sexual structures and temperature-growth ranges have been traditionally used for the identification of different *Pythium* spp. (van der Plaats-Niterink 1981; Dick 1990). However, it is frequently the case that most isolates are not identified to species level because of the time and experience needed to determined species. Recently, diagnosis has been complemented with molecular characterizations based on nucleotide sequences of the ITS of the rDNA (Lévesque and Cock 2004). In general, *Pythium* spp. are easy to isolate by using different methods. They are fast-growing species when cultured on potato-carrot agar at 25°C (van der Plaats-Niterink 1981).

Epidemiology

Pythium species occurs abundantly in cultivated soils near the root region in superficial soil layers. Most species of *Pythium* forms zoospores in water making them a potential threat in irrigation systems, and they also may be distributed by man and animals. Sexual reproduction structures are efficiently produced as most of the species are homothalic, being the oospores important survival structures.

Control

Darvas (1979) confirmed that high concentrations of metalaxyl applied to the soil eliminated *Pythium* spp. However, Darvas and Becker (1984) confirmed later that among *Pythium* species there are different degrees of susceptibility against the fungicide and that after its prolonged use there is a loss of efficacy which maybe caused by the development of resistance of the pathogen against the fungicide. In a broad sense, considering the physiological and pathological proximity of *Pythium* and *Phytophthora* species, similar control measures reported for Phytophthora root rot may be initially adapted for Pythium root rot.

Rosellinia root rot

The disease is also known as Dematophora root rot or white root rot of avocados. It was first reported in California by Raabe and Zentmyer (1955). The disease can be especially damaging under favourable environmental conditions for the development of the fungus, as occurs in southern Spain, where Rosellinia root rot is a limiting factor of the avocado crop (Pérez-Jiménez *et al*. 2005).

Symptoms

It is important to highlight that symptoms of plants infected by *R. necatrix* or *Armillaria* spp. are quite similar. This resulted in the initial identifications of the fungi involved in the root rot diseases being inaccurate in many cases. The first observable symptom of Rosellinia root rot is the existence on root surfaces of a white-cottony mycelium and mycelia strands coloured white or black. The fungus progresses by penetrating and rotting the tissue. In advanced stages of the disease, it is located between the bark and the wood, developing typical white mycelial fans which invade the entire root system causing a general rotting (**Fig. 10**). The evolution of aerial symptoms can occur quickly or slowly. In the first case, i.e. apoplexy, the tree suddenly declines in vigour; the leaves wilt and dry, and the tree dies during the next few weeks. These symptoms usually occur after a period of water or physiological stress. In the second case, symptoms developed over a period of several years. The tree shows retarded growth, sparse foliage, with wilting of leaves, chlorosis and death of twigs, branches and leaves. These symptoms worsen every year and when moisture and temperature are favourable the tree eventually dies (Pérez-Jiménez 1997).

Fig. 10 Adult avocado tree death caused by the infection of roots by *Rosellinia necatrix* (**A**). Symptoms of the pathogen on large roots may be naked eye observed as typically white mycelial strands developed under the bark (**B**).

Causal agents

Rosellinia necatrix (anamorph *Dematophora necatrix*) is an ascomycete soil fungus which has a worldwide distribution and is very aggressive and polyfagous; there are descriptions of more than 197 different hosts of this pathogen (Pérez-Jiménez 2006). On culture media, young mycelium of *R. necatrix* is white and cottony and with age it acquires a brown-black colour. In general, *R. necatrix* is a fast growing fungus and its mycelium covers all the surface of the culture medium when it is incubated in the dark at 20-24°C. The most relevant morphological characteristic of *R. necatrix* hyphae is the existence of pear-shaped or pyriform swellings immediately above the septum (**Fig. 11**). Generally, these swellings have been used as identification criteria for the species. *R. necatrix* may form sclerotia which are black, hard and spherical nodules or crusts located mainly on invaded roots and connected from their base with the subcortical mycelium. These structures, as in other fungi, seem to be related to the survival of *R. necatrix* in the soil.

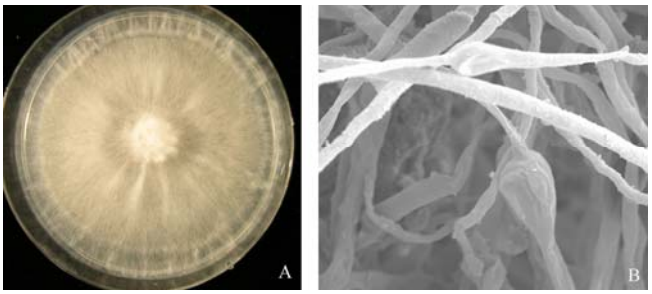

Fig. 11 Colony of *Rosellinia necatrix* growing on PDA (**A**). Typical pear-swellings as observed under electron microscope (×2000) (**B**).

R. necatrix produces three different spore types: chlamydospores, formed from pyriform swellings; conidiospores, originating at the end of the synnemata conidiogenous cells (length: 3.0-5.0 µm, width: 2.5-3.0 µm), and ascospores which are ellipsoidal and boat-shaped (length: 36.0-46.0 µm, width: 5.5-6.3 µm) (Petrini 1993; Sivanesan and Holliday 1985).

Epidemiology

The infective cycle of *R. necatrix* is basically underground and dispersal occurs in the soil, mostly through the contact between diseased and healthy roots of trees and further penetration of woody roots by mycelial strands. The fungus has a high saprophytic ability and can survive for long periods on dead wood (Pérez-Jiménez 2006). The life cycle of *R. necatrix* is limited to subterranean parts, which marks a difference with Armillaria root rot, which can develop white mycelial sheets above the plant crown. The different spores – chlamydospores, conidia and ascospores – seem to play a secondary role in the infective cycle of the fungus.

Control

Control of the fungus is difficult; however, there are some possible cultivation treatments that may prevent the spread of infection. Before planting, it is essential to remove plants, stumps and roots completely from all sites where the fungus has been present and to use healthy plants from nurseries. After planting, is important to avoid water stress, soil drench or drought, and if attacks do occur, to remove all diseased plants and to isolate diseased areas in order to prevent contact between infected and healthy roots. In seriously infested areas, it is recommended that resistant crops be grown (Abraham Sztejnberg, pers. comm.). Soil solarization was effective in the control of the white root rot in avocado orchards located in Spain because of the extreme susceptibility of this pathogen to temperatures above 30°C (López-Herrera *et al.* 1998). Is important to point out that most-used commercial *P. cinnamomi*-tolerant rootstocks such as 'Duke-7', 'Thomas' or 'Toro Canyon' have been confirmed to be highly susceptible to *R. necatrix* under artificial inoculations (Pérez-Jiménez *et al.* 2003). Commercial rootstocks tolerant to this pathogen are not available, although efforts are being made in Spain to develop them (Barceló *et al*. 2007). Studies related to the effect of different fungicides such as benomyl, tiophanate-methyl or fluazinam revealed certain effectiveness when they were applied as a soil drench under greenhouse conditions (López-Herrera *et al.* 2003). Biological control of the disease utilizing *Trichoderma* spp. has been confirmed in greenhouse experiments under artificial inoculations (Ruano Rosa 2006). Cazorla *et al*. (2006) correlated the production of 2- Hexyl 5-Propyl Resorcinol by *P. fluorescens* PCL 1606 isolated from avocado roots with biocontrol activity. Martín-Sánchez *et al.* (2007) selected antagonistic strains of *P. chlororaphis* from avocado roots with significant biocontrol capacity against *R. necatrix* and *P. cinnamomi*, main pathogens of avocado orchards in Spain. Pliego *et al.* (2007) selected strains of *P. pseudoalcaligenes* and *P. putida* from avocado rhizosphere which efficiently colonize and protect avocado roots.

Scab

This disease is important in areas with significant rainfall such as Florida, Mexico, Peru, Philippines and South Africa (Jenkins 1934; Ploetz *et al.* 1994; Téliz 2000). Lesions occur on leaves, stems or fruits.

Symptoms

On the fruit surface the fungus causes brown, corky, raised, oval-shape or irregular spots and lesions (Ploetz *et al.* 1994). As the disease extends, spots may coalesce giving a rough russet appearance to the whole fruit surface. The fungus does not penetrate the flesh but lesions may facilitate the entry of other fruit pathogens. On leaves, small (3 mm) dark brown spots develop along leaf veins (Jenkins 1934). Severe infections may crinkle and distort the leaves. Scabby large lesions may occur on leaf veins, pedicels and twigs.

Causal agents

Scab is caused by *Sphaceloma perseae*. On infected tissues, the fungus develops white, cream or olive masses of conidiophores (length: 25-100 µm) bearing hyaline conidia ovoid, 5-8 x 3-4 µm, or elongate coloured conidia 1-6 celled, reaching 30 × 3-5 µm. Germination occurs by hyaline sprout conidia or by germ tubes (Jenkins 1934). *S. perseae* may be isolated from tissues on PDA. The colour of the colony is variable from olive to brownish olive (Ploetz *et al.* 1994).

Epidemiology

Fungal lesion development needs high humidity and temperatures; in contrast, conidia may be formed throughout the whole year, although they are more abundant at the end of the infection period. Conidia are produced on infected tissues and spread by wind, rain or insects. In Mexico, populations of thrips (Thysanoptera) correlates with the number of lesions on fruits, since wounds caused by thrips favour the establishment and development of the fungus (Téliz 2000). Young fruits and leaf tissues are susceptible whereas mature ones are resistant (Ploetz *et al.* 1994).

Control

It is very important to eliminate infected fruits from the orchard because they are the primary inoculum source. Trees should be aerated by pruning and infected stems removed. As scab and thrips populations are positively correlated, thrips control may help to control the disease (Téliz 2000). Among cultivars, there are differences in susceptibility to scab. 'Lula' which is very susceptible, is not recommended for areas conductive for the disease. 'Fuerte', 'Hass', 'Booth 7' and 'Booth 8' are moderately susceptible whereas 'Pollack' and 'Waldin' are quite resistant. Chemicals should be applied to protect the fruit, particularly when humidity is above 60%. Sprays of copper fungicides are required for an effective control of scab. In Florida, it is recommended that spays are applied when bloom buds opens (late January), near the end of the main bloom period (mid February-March), and 3-4 weeks after the fruit has set (Ploetz *et al*. 1994).

Stem-end rot

The disease designated as stem-end rot is actually caused by diverse agents, and the name thus in fact integrates different diseases with similar symptoms. However, as it is referred to in the literature as a single disease, here the same concept is maintained. This disease is an economically important postharvest disease in most avocado-producing areas (Darvas and Kotzé 1981; Hartill 1991; Ploetz *et al*. 1994).

Symptoms

Stem-end rot is initiated as dark-brown to black rot at the stem end of avocado fruit. As the fruit ripens the rot progresses throughout the fruit into the flesh. Under high humidity conditions, fungal mycelium may develop on the fruit surface. Some fungal species causing stem-end rot may discolour vascular tissues in the advance front of the rot.

Causal agents

Stem-end rot is caused by several species of different fungal genera, the most commonly cited being *Colletotrichum*,

Botryosphaeria and its associated anamorphs, *Fusarium*, *Nectria* or *Phomopsis* (Darvas and Kotzé 1981; Hartill 1991). *C. gloeosporioides*, which was previously described under the anthracnose section, can cause stem-end rot alone or in combination with other pathogens. In the USA, the principal avocado stem-end rot pathogen reported is *B. dothidea* (anamorph *F. aesculi*). Meanwhile, in Australia and New Zealand the anamorphs *N. luteum* (syn. *F. luteum*) and *N. parvum* (syn. *F. parvum*) seems to be the primary pathogens related to stem-end rot. In South Africa, beside *N. luteum* and *N. ribis* (syn. *F. ribis*), *Nectria pseudotrichia* have also been described as causing the disease. In Israel, *B. rhodina* (anamorph *L. theobromae*) is the main cause of stem-end rot. Other species of fungi related with the disease are: *Alternaria* sp. *Fusarium decemcellulare*, *Phomopsis perseae*, *Pestalotiopsis vesicolor* and *Rhizopus stolonifer* (Darvas and Kotzé 1981). Most relevant species have previously been described.

Epidemiology

The species of fungi associated whith the disease occurs as endophytes or phellophytes in avocado stems, thus they can infect fruit from endophytically-colonized tissues. Pycnidia or ascomata may be produced on dead wood, leaves or fruits. Conidia or ascospores are spread by rain or wind to fruit or pedicel, initiating the infection. Infection can also be initiated during harvesting through the cut surface of the fruit (Hartill and Everet 2002). In general, the infection develops when the fruit ripens (Darvas and Kotzé 1981; Hartill 1991; Ploetz *et al*. 1994).

Control

To reduce preharvest infections, is important to remove dead wood or old fruits in order to reduce inoculum sources. Harvesting should be done in dry periods since wet conditions favour germination of spores and latent infections. Sterilising the clippers used for harvesting reduces the incidence of stem-end rot infections (Hartill and Everet 2002). Trees stressed by drought or nutrient deficiency are more susceptible to the disease thus it is important to maintain good tree vigour. Preharvest sprays with copper fungicides such as copper oxychloride (0.25%) and copper hydroxide (0.15%) alone or in combination with other fungicides such as captafol (0.16%) (Darvas and Kotzé 1981) give some control of stem-end rot. *Bacillus subtilis*, which has been used as a biocontrol agent against important post harvest diseases in South Africa, as commented on in the anthracnose section (Korsten *et al*. 1991, 1998), has been confirmed to attach to conidia and hyphae of important stem-end rot pathogens causing cell degradation (Demoz and Korsten 2006).

Verticillium wilt

Verticillium wilt of avocado was first described by Zentmyer (1949). Initially, in California the disease was called apoplexy or asphyxiation. It is not a serious disease although it has been recognized in several growing areas. The disease is usually found affecting isolated trees.

Symptoms

In affected trees the leaves suddenly wilt on one or several branches, or on an entire tree. The leaves rapidly turn brown but remain attached to affected branches for several weeks. If the bark is removed, brown to grey-brown streaks may be found in the wood. Frequently, affected trees will send out new shoots and the tree recovers completely.

Causal agents

Verticillium wilt of avocado is caused by *Verticillium dahliae*. This soilborne fungus is polyfagous with worldwide distribution. It is difficult to isolate from infected tissues, however semi-selective medium for *V. dahliae* have been described (Ausher *et al.* 1975). On PDA, *V. dahliae* produces hyaline elliptical to subcylindrical conidia (2.5-8 × 1.4-3.2 µm). They are formed at the tips of the phialides (conidiophores). Melanized microsclerotia consisting of clusters (50-200 µm) of rounded cells are also formed on PDA (**Fig. 12**). They are resistant structures which may survive in the soil for a long time under unfavourable conditions.

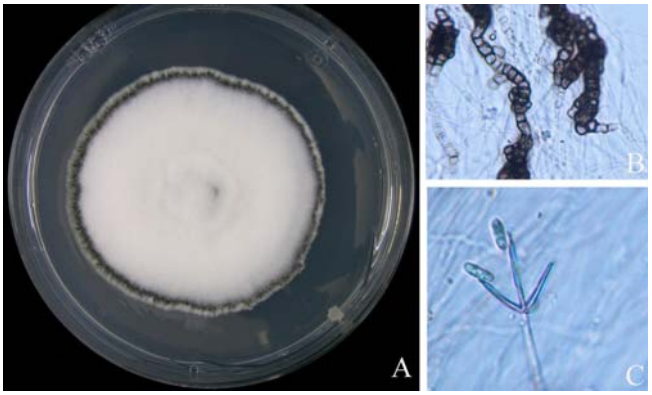

Fig. 12 Morphology of *Verticillium dahliae*. Colony growing on PDA (**A**). Microsclerotia consisting of clusters of melanized rounded cellls (×1000) (**B**). Conidia formed at the tips of phialides (×1000) (**C**).

Epidemiology

The probability of the disease is highest when avocado is planted in areas in which other hosts of *V. dahliae* have been grown. Microsclerotia of *V. dahliae* may survive in the soil for long periods as resting structures. The fungus is dispersed by water, wind and infected organic matter. The pathogen infects avocado roots often in spring after heavy rains, the mycelia invade upwards the xylem producing abundant conidia and the vessels may be plugged causing canopy wilt (Zentmyer 1984).

Control

It is not recommended to plant avocados on land in which important hosts of *V. dahliae* such as tomato, potato, strawberry or olives have been grown, nor to interplant susceptible crops (Zentmyer 1984). For rapid and accurate assessment of soil contamination by *V. dahliae* useful molecular techniques have been developed (Pérez-Artés *et al.* 2005). The existence of considerable differences in the sensitivity of avocado rootstocks to Verticillium wilt has been confirmed, Mexican rootstocks being more resistant to the disease than Guatemalan (Halma *et al.* 1954; Ben-Ya'acov and Frenkel 1973). Usually, no special treatments of affected trees are necessary but dead branches should be pruned after dieback has stopped and new growth begun.

FUTURE PERSPECTIVES: EMPHASISING A HOLISTIC APPROACH TO AVOCADO HEALTH

In the XXIst century a revolution of agricultural sciences is occurring because of changing priorities amongst the general public and policy makers. Agricultural production and processing practices need to be sustainable addressing health, environmental and philosophical concerns, whilst maintaining productivity and profitability in the face of constrains designed to preserve our natural resources. In this context, the holistic perspective considers the management of the biotic and abiotic constrains of the plant health through an integration of biological, physical and chemical treatments.

In this present work important diseases of avocado caused by fungi and oomycetes have been outlined. Some

have been presented because of their economic and global importance, others have been included due to the importance of their causal agents on significant agricultural or natural ecosystems. Although they have been presented as separate elements, is important to consider that the real situation may be that in which different organs of an unbalanced avocado tree are simultaneously co-infected with two or more aggressive pathogens coexisting with a crowd of non-lethal or opportunistic pathogens. Thus, avocado health research must also lead on managements strategies that can be readily integrated into holistic plant health maintenance systems, through the development of disease-management schemes that minimize the use of pesticides and maximize biological, genetic and cultural controls.

Significant principles of this wholistic approach have been exposed throughout this paper. The maintenance of soil organic matter, the conservation or enrichment of populations beneficial for the tree health, the use of clean seeds or plants thought the development of certified systems, the selection of rootstocks and cultivars adapted to local conditions and resistant to significant diseases, the reduction of nutritional and environmental stresses and the most common treatments with chemicals as strategies developed for avocado diseases control have all been commented upon. Moreover, most of them are integrating traditional methods with modern biotechnological approaches. Actually, important advances in the field of diagnosis based on PCR methodologies, in genetic resources assisted by *in vitro* tissue culture technologies, or in the development of integrated pest cycles and disease development supported by growth, pomology and productivity models are being achieved. Over the last decades, the control of avocado diseases has lead mainly on the use of chemicals and fungicides against established pathogens. It is likely that over the next decades the key element in the advance of avocado pathology will be the ability to integrate knowledge from other disciplines into a holistic approach toward the diagnosis and management of avocado diseases.

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