



# MAIN DISEASES OF AVOCADO IN BRAZIL

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## ABSTRACT

There may be many factors contributing to reduction in longevity and productivity in avocado trees, especially diseases of fungal and fungal-like pathogens, among which *Phytophthora* root rot, scab, cercospora spot, anthracnose, branch canker and stem-end rot are the most important. *Phytophthora* root rot is the most important disease of avocado. The pathogen attacks the fine feeder roots of avocado plants and induces multiple symptoms, and, if left untreated, can result in the death of trees and high economic losses. Scab and cercospora spot mainly affect fruit quality and cause fruit drop in intense attacks. Anthracnose and stem-end rot cause severe losses under favorable environmental conditions and the absence of preventive control, occurring mainly in postharvest, during storage and transport of the fruits. Branch canker affects the seedling set and reduces the productivity of the orchard, which can even cause the death of trees in severe attacks. The permanent occurrence of diseases in some areas may turn the crop unprofitable, which requires knowledge and adequate management to obtain productivity and high quality fruits. This review covers the main avocado diseases, their symptoms, etiology, epidemiology and management.

**KEYWORDS:** *Phytophthora cinnamomi*, *Sphaceloma perseeae*, *Pseudocercospora purpurea*, *Colletotrichum* spp., *Botryosphaeriaceae*

## RESUMO

Muitos fatores podem contribuir para a redução da longevidade e produtividade do abacateiro, especialmente doenças fungicas e causadas por patógenos semelhantes a fungos, entre os quais a podridão radicular de *Phytophthora*, a verrugose, a cercosporiose, a antracnose, o cancro de ramos e a podridão peduncular são os mais importantes. A podridão radicular de *Phytophthora* é a doença mais importante do abacateiro. O patógeno ataca as raízes finas do abacateiro e induz múltiplos sintomas e, se a doença não for tratada, pode resultar na morte de árvores e grandes perdas econômicas. A verrugose e a cercosporiose afetam principalmente a qualidade dos frutos e causam queda dos frutos em ataques intensos. A antracnose e a podridão peduncular causam perdas severas em condições ambientais favoráveis e na ausência de controle preventivo, ocorrendo principalmente na pós-colheita, durante o armazenamento e o transporte dos frutos. O cancro de ramos afeta o pegamento das mudas e reduz a produtividade do pomar, podendo causar até a morte das árvores em ataques severos. A ocorrência permanente de doenças em algumas áreas pode inviabilizar o pomar, o que requer conhecimento e manejo adequado para obtenção de produtividade e qualidade dos frutos. Esta revisão aborda as principais doenças do abacateiro, seus sintomas, etiologia, epidemiologia e manejo.

**PALAVRAS-CHAVE:** *Phytophthora cinnamomi*, *Sphaceloma perseeae*, *Pseudocercospora purpurea*, *Colletotrichum* spp., *Botryosphaeriaceae*

## PRINCIPAIS DOENÇAS DO ABACATEIRO NO BRASIL

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## INTRODUCTION

Avocado (*Persea americana* Miller) is a significant and nutritious fruit crop grown in both the tropic and subtropical regions in many parts of the world. The Brazilian and world demand and production of avocados have been growing in recent years, with Brazil being responsible for 243 thousand tons produced in 2019, occupying the seventh position in the world with 3.4% of the avocado produced, with the State of São Paulo being the main producer with 123 thousand tons (AGRIANUAL 2022).

Three botanical races are distinguished from the avocado: the Mexican race (*P. americana* var. *drymifolia*), the Guatemalan race (*P. americana* var. *guatemalensis*) and the Antillean race (*P. americana* var. *americana*). The differences between them lie mainly in fruit size and shape, texture, shell thickness and color, seed size and fruit maturity, and nutraceutical potential (BERGH 1992). Most commercial avocado varieties are interracial hybrids developed from the exchange of materials between the different races. Among the varieties of avocado planted in Brazil are Hass, Breda, Fortuna, Fuchs, Geada, Margarida, and Quintal (PICCININ *et al.* 2016).

Avocado growers face several challenges for growing sustainable fruits among which, disease management stands out. Understanding the interaction of pathogens with their avocado host is critical to the development of disease management options. Important factors include the presence or absence of a particular pathogen, the susceptibility of the avocado cultivar or rootstock, the optimum environmental conditions for disease development, and the level of disease that will likely occur in a given situation. Diseases can significantly affect productivity and fruit quality, even causing the death of plants if not properly managed and resulting in significant economic losses that can derail the viability of the orchard.

## PHYTOPHTHORA ROOT ROT

Phytophthora root rot (PRR), caused by *Phytophthora cinnamomi* Rands, is the most serious and important disease of avocado worldwide. It attacks trees of all ages, by destroying the fine feeder roots and without intervention, affected trees eventually die. *P. cinnamomi* is now known to affect more than 5,000 plant species (HARDHAM & BLACKMAN 2018).

## Symptoms

Infection is mostly limited to the fine feeder roots, responsible for supplying water and nutrients, and which become black and brittle and eventually die (Figure 1A) (ZENTMYER 1980). As the disease progresses, the pathogen may invade and destroy the entire feeder root system. Feeder roots may be very difficult to find under trees with advanced symptoms of root rot. Beneath such trees the soil tends to be abnormally wet, as destruction of feeder roots prevents the uptake of water. Larger woody roots are rarely infected and remain functional until the tree dies. Trunk cankers may form occasionally, being more common on Mexican rootstocks (DANN *et al.* 2013).

Besides reduced uptake of water and the steady depletion of carbohydrates, visible symptoms in the tree can also result from interference with mineral uptake and distribution of nutrients in plant tissue. Leaves become chlorotic and wilted (Figure 1B) and often have necrotic tips and margins and may abscise prematurely. There is a significant reduction in vegetative growth resulting in a reduced tree canopy (Figure 1C). Entire limbs may die back. If leaves do form, they are small and pale green. Fruit yields decline in root rot affected trees but sometimes such trees set a large number of small fruits. In flooded soils, avocado trees may collapse suddenly with wilted leaves hanging on the tree for several weeks. This 'rapid death' syndrome in wet soils can be solely due to anaerobic conditions (STOLZY *et al.* 1967) or hypoxic soil conditions from temporary flooding exacerbated by PRR (PLOETZ & SCHAFFER 1989).

## Causal agent

The soil-borne pathogen *P. cinnamomi* is a member of the class Oomycetes, Chromista Kingdom, and forms fungal-like *nonseptate* hyphae and conidia-like sporangiospores, but it is not a fungus. It forms three different types of propagules that are involved in disease development or survival: sporangia, zoospores and chlamydospores (ZENTMYER 1980). Sporangia, which germinate directly with germ tubes or indirectly by producing motile zoospores, are nonpapillate and non-caducous (persistent); and elliptical to ovoid. Average measurements of sporangia range from 43–75 mm long to 24–47 mm wide, with an average length:breadth ratio of 1.54. New sporangia are produced by internal or ex-



**Figure 1.** Symptoms of *Phytophthora* root rot on avocado. Healthy avocado roots (left) and with *Phytophthora* root rot (right) (A). Avocado seedling with shoot symptoms after inoculation with *Phytophthora cinnamomi* in the root system (B). Avocado tree with *Phytophthora* root rot symptoms (right) and healthy plant (left) (C).

ternal proliferation or by sympodial development of the sporangiophore immediately below empty sporangia. Sporangia do not form readily in sterile culture but are produced in nonsterile soil extract (10 g soil l<sup>-1</sup>) or in sterile salt solution (CHEN & ZENTMYER 1970).

Chlamydospores form readily in culture (on V8 juice agar or V8 juice broth) and in infected plant tissues; sizes range from 31–50 μm in diameter. They are either terminal or intercalary in the mycelium and usually occur in grapelike (botryose) clusters. *P. cinnamomi* also produces clusters of hyphal swellings giving a distinctive coralloid appearance to the hyphae on V8 juice agar or selective media. When cultured on potato dextrose agar (PDA), colonies have a distinctive camellioid or rosette pattern (DANN *et al.* 2013).

*P. cinnamomi* is diploid in its vegetative state and heterothallic (outbreeding), possessing A1 and A2 compatibility types or mating strains (GALINDO & ZENTMYER 1964). Oospores are sexual spores with thick walls and are formed when strains of opposite compatibility are paired. The A2 mating type sometimes forms homothallic (selfing) oospores when incubated in avocado root extracts (ZENTMYER 1979). The A2 mating type predominates in avocado orchards worldwide. The cardinal temperatures for growth are 5–15°C, 20–32.5°C and 30–36°C (ZENTMYER 1981; DANN *et al.* 2013).

*P. cinnamomi* can be readily detected using traditional methods such as direct isolation by plating diseased tissue on to selective media such as P10VP, which contains 10 ppm pimaricin, 200 ppm vancomycin, 100 ppm pentachloronitrobenzene (TSAO & OCANA 1969) amended with 50 ppm hymexazol, or baiting with susceptible plant tissue from infected soil. More rapid and specific antibody

and DNA tests have been developed in recent years. A nested real-time PCR allowed specific, sensitive, and quantitative detection of *P. cinnamomi* within root tissues of avocado (ENGELBRECHT *et al.* 2013), even allowing to distinguish avocado rootstocks as resistant or tolerant during avocado infection (VAN DEN BERG *et al.* 2021).

### Epidemiology

The disease cycle usually begins with infected feeder roots or chlamydospores producing sporangia in warm, moist and aerated soils (ZENTMYER 1980). When soil pores are full of water each sporangium produces up to 40 motile biflagellate zoospores. These zoospores are chemotactically and electrostatically attracted to the soft tissue at the zone of root elongation just behind the root caps. They then shed their flagella and form cysts that produce germ tubes that secrete cell wall degrading enzymes and penetrate the root cells. Once within the host cell, the germ tube proliferates and branches to form mycelium which is found throughout the feeder root within 72 h (ZENTMYER 1980). If the avocado tree does not detect the presence of the pathogen, the mycelium colonizes the entire root. If the plant detects the presence of the pathogen, it reacts quickly by producing biochemical defenses such as deposition of phenolic compounds (AVELING & RIJKENBERG 1989) and undergoes hypersensitive cell death (PHILLIPS *et al.* 1987) that successfully inhibits disease development. If ingress is not inhibited, sporangiophores will emerge from the root surface and another generation of sporangia are produced. The zoospore to zoospore cycle may take as little as 24 h. If favourable environmental conditions prevail there is a rapid increase in the population leading to an exponential build-up of epidemic disease (DANN



et al. 2013).

Chlamydospores, which form prolifically in decaying roots, survive for considerable periods in root fragments and dry soil, with their viability reaching up to eight years in the absence of the host (PICCININ *et al.* 2016). They germinate directly by producing several germ tubes, or indirectly to form sporangia when soil is briefly saturated. The pathogen also has good saprophytic capacity, being able to survive for long periods in this way. Oospores, which are also survival spores, occur infrequently and probably do not play a major role in the disease cycle.

Soil moisture is the major environmental factor influencing disease development. Water-filled soil pores are required for zoospores to release, disperse and find their way to feeder roots. However, *P. cinnamomi* is an aerobic organism and does not tolerate low oxygen levels in soil. Contrary to common belief, it is not favoured by waterlogged soils when conditions are anaerobic. Short periods of soil saturation with aerated water favour *P. cinnamomi*, whereas prolonged periods of soil saturation lead to anoxia (i.e. waterlogging) which will damage or kill avocado roots and inhibit the pathogen. Disease develops optimally at temperatures between 19–25°C and declines at > 30°C and < 12°C. Root rot is most severe at lower temperatures, where *P. cinnamomi* grows better than avocado trees, and is much reduced at higher temperatures that favour the host. Disease develops over a wide range of pH (3.5–8.0) (DANN *et al.* 2013).

Flooding, drought and salinity can predispose avocado roots to infection presumably due to increased root exudates. When roots stressed by salinity are infected by *P. cinnamomi* they lose the ability to exclude salt and leaf tips and margins become necrotic, which is a typical symptom of salt and specifically chloride damage (WHILEY *et al.* 1987).

The pathogen spreads to new areas in infested soil, water, and by human and animal activity. It also spreads by zoospores and small pieces of infested plant tissue in surface flow on saturated land and very occasionally by seeds taken from infected fruit in contact with soil. *P. cinnamomi* is very commonly spread in infected nursery trees.

## Management

### Site selection

Trees should only be established in soils that have good internal and surface drainage. Soils should be at least 1.5 m in depth and water should be able to flow constantly through them during the heaviest expected rainfall. Soils with impervious subsoil layers, or with high clay content, are more likely to allow water to accumulate around plant roots and therefore favour disease development. Since soil salinity is a major stress factor for avocado, saline soils should be avoided when selecting a site for an avocado orchard. Planting on mounds or ridges (1–1.5 m wide and 0.5–1 m high) can be used to reduce temporary soil saturation and ensure that roots are in friable, aerated soil. Mounds should be formed with the slope of the land and, if needed, drains should be constructed across the slope to prevent erosion (DANN *et al.* 2013).

### Producing healthy nursery trees

To prevent the introduction of *P. cinnamomi* with seed, it must be collected and processed so that it does not have contact with the soil; otherwise, seeds should be treated with hot water (49–50°C for 30 min; 52°C damages the seed) prior to planting. Close attention to hygiene procedures will minimize or eliminate *P. cinnamomi* and other pathogens. These procedures include secure perimeter fencing with controlled access points where staff must walk through a footbath containing a copper fungicide. Plants should be held on steel mesh benches at a height of at least 1 m above the nursery floor to allow free drainage from the bottom of the nursery bags. The nursery floor should be of bitumen, concrete, gravel or crushed rock and this can be regularly treated with copper fungicides or chlorinated water. Irrigation water should come from a clean reticulated water supply or from deep wells or bores. Water from rivers, canals and dams is often contaminated with *P. cinnamomi* and must be disinfested with chlorine, considering a minimum residual chlorine level of 0.5 mg/mL<sup>-1</sup> after treatment. Vehicles and equipment should be thoroughly cleaned to remove all soil and plant debris and then disinfested before entering the nursery. Affected plants must be destroyed (DANN *et al.* 2013).

## Nutrition and irrigation

A balanced nutritional programme, based on soil and leaf analysis, will produce a good summer growth flush and promote feeder root development during the autumn and winter months. The addition of calcium, magnesium, manganese, phosphorus and silicon ions to avocado crops has shown a positive effect on PRR disease control (RAMÍREZ-GIL *et al.* 2017; RAMÍREZ-GIL & MORALES-OSORIO 2020). High levels of nitrogen and pH increase the plant's predisposition to disease (PICCININ *et al.* 2016).

A well-designed irrigation system is an integral component of a PRR control programme. As most feeder roots are found in the top 45 cm of soil it is necessary to keep the upper root zone moist at all times through irrigation and mulching. For correct irrigation, growers should vary water applications depending on readings from soil-based monitoring systems (e.g. tensiometers or soil capacitance probes) or based on local evaporation demands. Trees must not be over-watered (soil suction of 0–10 kPa) or under-watered (soil suction of 50–70 kPa) as these conditions predispose roots to *P. cinnamomi* infection (DANN *et al.* 2013).

It is difficult to manage irrigation in orchards where some trees show decline from root rot. These trees, with substantially fewer healthy feeder roots for uptake of water, will be overwatered with further loss of roots if irrigation scheduling is based on soil water content values near healthy trees. This can be accounted for by replacing minisprinklers under declining trees with lower output sprinklers.

## Soil solarization

Soil solarization involves trapping radiant heat from the sun under clear polyethylene sheets that have been laid on the surface of the soil to increase soil temperatures above 45°C in the top 5 cm of soil. Preplant solarization is particularly effective for treating infested soil in countries with hot summers and cloud-free days. It reduces, but does not eliminate, root rot (GALLO *et al.* 2007).

## Cultural control

Calcium applied to some soils can reduce PRR and increase yields by improving soil drainage and aeration. It also has mild fungicidal properties, acting directly on the reproduction of the pathogen. Gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) added to soil in avocado orchards in California reduced sporangial production

and size and zoospore numbers, thus reducing disease pressure (MESSENGER *et al.* 2000). In eastern Australia, annual applications of gypsum ( $10 \text{ t ha}^{-1}$ ) over a 6-year period to a 'Fuerte' orchard infected with *P. cinnamomi* increased yields by 40% compared to trees that received dolomite (BROADBENT *et al.* 1989). This increased yield was probably due to the fungicidal properties of gypsum as a direct nutritional effect did not appear to be involved. As excessive amounts of soil-applied gypsum can displace K and Mg there is a need to regularly monitor levels of all nutrients to maintain tree health and fruit quality.

Positive results have already been observed with potassium silicate in areas affected by the disease. Three successive drenches of soluble potassium silicate resulted in the most increase in root density and on canopy health (BEKKER *et al.* 2014). In another study, potassium silicate applications improved yield and fruit quality, but the visual health of trees in decline due to PRR was not affected, evidencing inconsistent effects of potassium silicate under field conditions (DANN & LE, 2017).

Animal manures, especially fresh or composted chicken manure, have been shown to suppress *Phytophthora* (BROADBENT & BAKER 1974; ARYANTHA *et al.* 2000). Manures release ammonia and organic acids which are toxic to *P. cinnamomi* but also to avocado roots. Chicken manure increases total biological activity and populations of antagonistic actinomycetes, fluorescent *Pseudomonas*, fungi and endospore-forming bacteria. It is best incorporated into the soil months prior to planting to reduce possible toxic levels of chloride and ammonia, or applied sparingly on top of organic mulches.

Excessive cincturing to increase yields can be detrimental where PRR is a significant problem and disease management is inadequate. Carbohydrate supply to roots is decreased, thus reducing the ability of the tree to replace roots lost to *P. cinnamomi* (DANN *et al.* 2013).

Anatomical and physiological incompatibility at the stock/scion interface can also influence the effect of root rot. Trees with scion overgrowth are particularly susceptible as phloem translocation to the roots is severely compromised and such trees are unable to replace *Phytophthora* affected roots (DANN *et al.* 2013).

## Systemic chemicals

Use phosphite, a neutralized solution of the phosphonate anion, when the disease is seen early. It moves rapidly in the xylem and are then remobilized in the phloem with photoassimilates in a source-sink relationship. Phosphites, which can be applied as soil drenches, foliar sprays, trunk paints or trunk injections, induce a strong and rapid defense response in a challenged plant. They have a complex mode of action, acting directly on the pathogen and indirectly in stimulating a host defense response to ultimately inhibit root colonization by the pathogen (GUEST *et al.* 1995). The pathogen is rarely killed and treatments have to be reapplied when phosphite concentrations fall below an effective level. Plants do not metabolize phosphite but tissue concentrations decline from: (i) dilution through plant growth; (ii) harvesting of fruit; (iii) loss in root exudates; and (iv) loss in senescing organs that abscise from the plant. Thus, critical concentrations in roots (> 25 ppm phosphate) must be maintained for disease control with appropriately timed sequential applications (DANN *et al.* 2013).

Soil drenches have proved ineffective. It was found that phosphite applied in this way did not persist in the roots and consequently it had to be reapplied monthly at high rates to maintain a critical root concentration. Trunk application is only effective on young avocado trees as phosphite will not easily penetrate the bark of older trees to provide an effective root concentration. Initial injection research concentrated on rejuvenating severely affected trees but later shifted from curative to preventative management. The most effective times to apply phosphite to maximize feeder root concentrations are in late spring when shoot growth has matured and again at the completion of the summer growth flush. At these two stages of phenological development, feeder roots are relatively strong sinks in the tree. Trees showing symptoms of root rot should be injected twice per growing season, whereas healthy trees are injected only once to achieve maximum levels and persistence of phosphite in feeder roots. This injection is made after summer leaf and root flushing are complete but before floral bud development. Since there is little lateral redistribution of phosphite across trunk tissues in avocado trees, injection sites must be evenly spaced around the trunk. Trees should be free of water stress when injected. The best time to inject trees is when they are

actively transpiring. High volume foliar sprays (0.5% a.i. (active ingredient) mono-dipotassium phosphonate, pH adjusted to 7.2) applied without surfactants to thoroughly saturate healthy trees (> 2500 l ha<sup>-1</sup>) will provide the same root concentrations as the injection programme. Four to six strategically timed sprays may be required (DANN *et al.* 2013).

In South Africa (DUVENHAGE 1994) and Australia (DANN *et al.* 2013), isolates of *P. cinnamomi*, which are less sensitive to phosphite *in vitro*, were recovered from beneath avocado trees which had been repeatedly injected or sprayed with phosphite. Phosphite selects out the least sensitive isolates from the naturally occurring diversity within *P. cinnamomi* populations. However, only tenfold reductions in sensitivity develop and effective concentrations of phosphite can still be maintained in roots to provide disease control in the presence of less sensitive populations of *P. cinnamomi*.

Concerns have been expressed that injections could cause trunk decay and damage (COFFEY 1992). Injection holes from low pressure injections callus rapidly without fungal colonization, but a brown staining of the wood above and below the injection site remains, perhaps caused by the accumulation of phenolic compounds in the xylem vessels. However, there is no evidence that this is detrimental to tree health or yield.

Metalaxyl fungicide has been effective against *Phytophthora* spp. if used following technical recommendations. The fungicide is absorbed by avocado roots, moves rapidly in the xylem and acts directly on pathogen to inhibit their growth and sporulation (FARIH *et al.* 1981). A single application of metalaxyl provides disease control for 3 months. However, after multiple applications in some avocado soils, especially those with high organic matter contents, its efficacy is reduced due to rapid biodegradation which reduces the half-life of the chemical from 12 weeks to as short as 1 to 2 weeks (AL-SA'DI *et al.* 2008). In addition, experimental evidence of reduced sensitivity and effectiveness after prolonged use has been reported for *P. cinnamomi*, suggesting that intensive and continuous applications should be avoided (DARVAS & BECKER 1984; BAILEY & COFFEY 1985; HU *et al.* 2010). The high activity and performance of oxathiapiprolin, fluopicolide and mandipropamid fungicides, more efficient than mefenoxam (R-enantiomer of metalaxyl) and potassium phosphite in controlling the disease in gree-

nhouse, supports their registrations on avocado for use in rotation and mixture programs, to reduce the risk of development and spread of resistance in pathogen populations (BELISLE *et al.* 2019). In Brazil, where there are no registered fungicides to control the disease (AGROFIT 2023), sensitivity of *P. cinnamomi* populations to metalaxyl and other chemicals used for its control is mostly unknown, indicating the importance of performing these analyses to develop a rational management strategy.

### Mulching and suppressive soils

The benefits of mulching to suppress PRR have been well documented (BROADBENT & BAKER 1974; WOLSTENHOLME *et al.* 1996; DOWNER *et al.* 2001). Mulching also affects the vigour of trees by reducing surface temperature, and improving the structure and moisture and nutrient-holding capacity of soil. Mulch promotes a complex and antagonistic soil microflora and fauna. *P. cinnamomi* is a relatively poor saprophytic competitor and struggles to exist in soils rich in organic matter. Suitable mulching materials include chipped avocado prunings, aged hardwood chips (for example eucalypts), aged or composted pine bark, wheat straw and sorghum stubble. Natural leaf fall is also beneficial but not as effective as woody mulches. These materials have a carbon:nitrogen (C:N) ratio between 25:1 and 100:1 and thus any serious nitrogen draw-down is avoided. Besides reducing root rot by increasing biological activity and biodiversity, mulching allows roots to proliferate in an environment relatively free of *P. cinnamomi*. In addition to root rot suppression, benefits include increased yields and fruit size. Since mulch reduces water loss, it is necessary to monitor soil moisture levels under mulch and vary irrigation schedules (DANN *et al.* 2013).

Soils suppressive to avocado root rot occur naturally. For example, established plantings on Australian subtropical rainforest soils derived from basalt (krasnozems) have some root infection but trees remain healthy. The Ashburner system (BROADBENT & BAKER 1974), designed to maintain a healthy avocado orchard in *P. cinnamomi*-infected soils, simulated the disease-suppressive rainforest soil by continually adding large amounts of plant residues from cover cropping, mulch, and chicken manure and dolomite to improve soil health and stimulate indigenous suppressive microbes. If extensive applications of organic amendments are not made

to these rainforest soils serious outbreaks of root rot occur in avocado orchards. In recent times, the Ashburner system has been modified with additions of coarse mulches to provide an oxygen-rich root environment, and gypsum. Light applications of chicken manure broadcast on the mulch are also occasionally made (DANN *et al.* 2013).

The introduction of specific antagonists such as *Trichoderma*, *Gliocladium*, *Bacillus* and *Streptomyces* to soils to control *P. cinnamomi* has given mixed results in field trials (ERWIN & RIBEIRO 1996). Successful examples of biological control of *Phytophthora* using this approach are limited to controlled nursery environments. Simply adding specific microorganisms to disease-conducive soils does not appear as effective as organic matter amendments to manipulate indigenous microorganisms for the suppression of PRR. Much more research is needed before the biological control of this disease with individual antagonistic microorganisms is commercially and economically feasible (DANN *et al.* 2013).

### Resistance

Host resistance is the best method for reducing PRR (COFFEY 1992; ZENTMYER 1994). Some resistant rootstocks produce new feeder roots more quickly than susceptible rootstocks in the presence of *P. cinnamomi*, while in others, colonization of the root is inhibited (PHILLIPS *et al.* 1987). This resistance is associated with attractiveness to zoospores in terms of either encystment rates or the concentration and composition of exudates produced by the roots; deposition of structural barriers such as callose and tyloses, and the induction of various biochemical defense pathways within avocado roots in response to *P. cinnamomi* infection (BOTHAS *et al.* 1990; BACKER *et al.* 2015; VAN DEN BERG *et al.* 2018).

Resistance in current rootstocks is not absolute and control still requires the use of traditional methods of management, particularly during the establishment of nursery trees in replanted land and where disease pressure is high. The range of resistant rootstocks has increased in recent decades in some countries with the release of some materials with greater tolerance/resistance, such as 'Dusa', 'Latas', 'Bounty', 'Zentmyer', 'Uzi', 'Steddom' and 'Velvick' (MENGE *et al.* 2012). Barr Duke, Duke 6, Duke 7, Duke 9, Thomas, and Toro Canyon can also be good rootstocks but have less *Phytophthora*-resistance than some newer cultivars. In Brazil, avoca-



do seedlings grafted on resistant rootstocks, such as Dusa, Velvick and Duke 7, are already commercialized by a few nurseries, and an increase in the supply of resistant rootstock is expected in the coming years. Future research utilizing molecular genetic technologies, such as molecular markers, will assist with the selection and generation of such material.

### Integrated control

A complete appreciation of the disease cycle and epidemiology is necessary when devising a management strategy for PRR. Because of the short generation time and high reproductive capacity of *P. cinnamomi*, the pathogen has the ability to cause massive fine feeder root death in wet or flooded conditions. Combating a pathogen of this type requires a combination of the practical management procedures designed to reduce pathogen activity and increase host resistance during critical infection periods, seeking economic production of avocado in the presence of *P. cinnamomi*. Management practices should include prevention (quarantine, nursery hygiene), cultural control (drainage, irrigation, addition of calcium), biological control (use of suppressive soils, mulching), chemical control and rootstock resistance (ERWIN & RIBEIRO 1996). Research suggests that individual control methods are not as effective as when they are applied in combination, such as treatment combining metalaxyl + mancozeb applied in drench; injection of potassium phosphite; potassium silicate applied in drench; addition of a layer of organic mulch and incorporation of 10 kg of composted substrate, both applied to the ground around the base of each tree, suggesting a synergistic effect (RAMÍREZ-GIL *et al.* 2017). Similarly, in another study, the most promising strategies included fungicide use (rotation of metalaxyl and phosphites), organic matter in the form of residues of vegetables and mushrooms, use of foliar (manganese) and soil (calcium, magnesium, phosphorus, and silicon) ions, additions of bovine manure, beneficial microorganisms (*Rhizoglyphus fasciculatum* (Thaxt.) Sieverd., G.A. Silva & Oehl, *Trichoderma* sp., and *Bacillus* sp.), edaphic application of auxin and an energy source (sucrose) (RAMÍREZ-GIL & MORALES-OSORIO 2020).

### SCAB

Scab is a serious problem in humid avocado growing regions, such as Florida, Latin and South America, Morocco and the Philippines. Severe losses

result from fruit drop and the lowered market value of affected fruit. It is also important in nurseries, affecting the initial development of plants (DANN *et al.* 2013; PICCININ *et al.* 2016).

### Symptoms

Fruit spots are initially oval to irregular in shape, 5 to 6 mm in diameter, brown to purple-brown, and slightly raised with a sandpaper-like surface (POHRONENZY & SIMONE 1994). As the disease progresses, spots enlarge and coalesce. Often there are intersecting raised ridges, and large rough, corky areas may form over the surface of the fruit (Figure 2). The infection in the fruits does not go beyond the skin. Fruit symptoms are very similar to those caused by wind rub and some insects (DANN *et al.* 2013).

Lesions on leaves are less conspicuous because they are often high in the tree canopy. They are rounded when located on the leaf blade and slightly elongated on the leaf veins, initially less than 3.5 mm in diameter and become necrotic and brown to black. They are often concentrated along leaf veins and cause leaves to become stunted, crinkled and distorted. Lesions may coalesce into star-like patterns and shot holes develop in the leaves. Raised, corky, roughened, oval to elongate lesions also occur on twigs and pedicels (DANN *et al.* 2013).



**Figure 2.** Symptoms of scab on avocado.

### Causal agent

Scab is caused by *Sphaceloma perseae* Jenkins. Acervuli erupt from leaves or fruit lesions as small, white, cream to olive masses of clustered conidiophores and spores. Conidiophores are 12 to



100 µm in length and bear conidia along their tips or sides. Conidia are colourless, aseptate, ovoid to strongly curved and 2–30 × 2–5 µm. On PDA, the fungus is slow growing and produces white to dark grey mycelium (JENKINS 1934; DANN *et al.* 2013).

### Epidemiology

During cool, moist weather, conidia may be formed on infected leaves, twigs and fruit. Sporulation in fruits starts at one month of age and gradually reduces until four months, when only a few conidiophores are observed (JENKINS 1934). Conidia are carried to infection courts by wind, rain and insects. In Mexico, most spores were produced in the winter prior to active tree growth, while most lesions were found six months later after the fruit set and foliage flush (TELIZ 2000). Infected seedlings spread the disease over long distances (PICCININ *et al.* 2016).

*S. perseae* is a pathogen of young tissue. Leaves become resistant one month after emergence, approximately 3 cm long, and fruit become resistant once they reach about half size (POHRONENZY & SIMONE 1994). Disease is most severe when host tissue is at a susceptible growth stage and heavy rains or foggy weather keep the humidity above 80% (TELIZ 2000). According to Ávila-Quezada *et al.* (2003), 'Has' avocado fruits are most susceptible at phenological stages 1 (3–7mm wide x 4–10mm long) to 3 (20–29mm wide x 24–39mm long). Injuries caused by thrips create entry wounds for *S. perseae* and greatly exacerbate scab development, also considering that the symptoms of thrips damage alone can be confused with scab in the early stages of the disease. The rind injuries that are caused by scab are often used as entry points by other pathogens. When other fruit diseases interact with scab, fruit quality drops dramatically (DANN *et al.* 2013). In unfavorable weather conditions, the fungus survives on infected leaves and remaining fruits. Only the avocado is reported as a host of the pathogen (PICCININ *et al.* 2016).

### Management

It is recommended to use varieties with some level of resistance or slightly susceptible to scab, such as Booth 1, Collins, Collinson, Ouro Verde, Quintal and Waldin. The varieties Booth 3, 5, 7 and 8, Choquette, Fuerte, Hass, Monroe, Pollock and Trapp are moderately susceptible, whereas

Geadá, Hall and Lula are very susceptible (POHRONENZY & SIMONE 1994; PICCININ *et al.* 2016; SILVA *et al.* 2022). In general, varieties from the Antillean race have high susceptibility to leaf scab and lower susceptibility to fruit scab, while varieties from the Guatemalan race, in turn, have high susceptibility in fruits and low susceptibility in leaves (PICCININ *et al.* 2016).

Scabby fruit, which are left on the tree during harvest, often become primary sources of inoculum the following year. Thus, scabby fruit should be removed from the orchard. Canopies should be pruned regularly and skirts removed to improve air movement and the penetration of sunlight (TELIZ 2000).

Sprays of copper fungicides should be made as flower buds appear, near the main bloom period, and 3–4 weeks later (POHRONENZY & SIMONE 1994). More sprays may be required during periods of heavy rain or fog, until the fruit reaches approximately 5 cm in diameter (*fruit half-grown*). If humidity remains below 60%, some of these sprays may be omitted (TELIZ 2000). In areas with higher occurrence of the disease, control can be done by alternating cupric fungicides with difenoconazole, also registered for the disease in Brazil (AGROFIT 2023). In seedling nurseries, for the varieties of the Guatemalan race, a biweekly application of copper fungicides must be carried out (PICCININ *et al.* 2016).

Effective thrips control must accompany any effective scab control program (Teliz, 2000). Planting windbreaks in orchards reduces the incidence of fruit injuries resulting from friction against other fruits, branches and leaves (PICCININ *et al.* 2016).

### CERCOSPORA SPOT

Cercospora spot of avocado is found globally in all subtropical and tropical regions with warm and humid climates, being important in the northern Australia, Brazil, Colombia, Florida (USA), Mexico, South Africa, Spain and West Indies (DANN *et al.* 2013; CABI 2016; RAMÍREZ-GIL *et al.* 2020; RODRÍGUES *et al.* 2020). Fruit losses due to cosmetic damage can be up to 70% in unsprayed orchards in the warm subtropics of South Africa (KALLIDEEN 2020).

### Symptoms

Symptoms occur on leaves, stems and fruit. Lesions first appear on the lower surface of leaves as

small (1–5 mm), angular, light brown to gray flecks or spots, usually with a chlorotic halo. Spots eventually become visible on both leaf surfaces and often coalesce to produce large, brown, dead areas on the leaf. Under high humidity, grey spore-bearing structures of the fungus are clearly visible on the spots (DANN *et al.* 2013). Necrotic tissue in the center of the lesions may fall out, facilitating the tearing of the leaf blade (PICCININ *et al.* 2016).

On fruits, lesions first appear as small dark spots which later become irregular brown blotches, slightly sunken, with defined edges and approximately 3 to 6 mm in diameter (Figure 3). Eventually the tissue dries out becoming cracked and fissured which may allow the entry of other pathogens such as *Colletotrichum* spp. Sometimes, infection is temporarily arrested and disease is manifested as minute (< 3 mm), raised, black, shiny spots often associated with cracking and corking of lenticels (DANN *et al.* 2013). The disease is usually superficial, but the flesh can be invaded during advanced stages of the disease. On green twigs and fruit pedicels, darkbrown to black irregular lesions (2–10 mm) can develop, causing premature fruit fall. In addition to the loss of fruit quality, fruit drop is one of the most severe symptoms of the disease, which can reduce production in very susceptible varieties (PICCININ *et al.* 2016).



**Figure 3.** Symptoms of cercospora spot on avocado.

#### Causal agent

Cercospora spot is caused by *Pseudocercospora purpurea* (Cooke) Deighton. It produces dark brown to black, spherical to irregular stromata, 15–125 µm in diameter, that are immersed in leaves and fruit. Conidiophores are 3–4.5 × 20–200

µm long, pale to olive brown, multiseptate, rarely branched, straight or with a zigzag growth, with small scars on the rounded tips where conidia have dehisced. Conidia are obclavate to cylindrical, with a truncate base, pale olive, indistinctly one to nine septate, straight to curved, and 2–4.5 × 20–100 µm. The fungus is thought to produce a *Mycosphaerella* teleomorph which is rarely found and considered unimportant in the disease cycle (DANN *et al.* 2013).

*P. purpurea* grows readily on standard media such as PDA. In a growth study, the oatmeal agar stood out with optimal growth temperature at 25°C (KALLIDEEN 2020). Isolations should be made from fresh young lesions following surface decontamination. It produces a tufted leathery growth which is initially grey, but later becomes brown to black.

#### Epidemiology

Although a teleomorph of the pathogen may exist, infection mainly occurs through conidia which are present on leaves all year-round, especially during warm, rainy weather when relative humidity is high. Conidia are spread easily by wind, splashing rain, irrigation water and insects to initiate new infections. Fruit between 1/4 to 3/4 full size are susceptible, whereas very young fruit and fruit near maturity are highly resistant. Penetration can be direct, or through wounds and after penetration, the pathogen remains latent for up to three months. Infections early in the season result in the highest disease incidence and severity at harvest (DANN *et al.* 2013).

*Persea americana* is the main host of the pathogen, also occurring in *P. borbonia* L. Spreng. (redbay) and *P. palustris* (Raf.) Sarg. (swamp bay) (CHUPP 1953).

#### Management

Since infection occurs in the developing fruits, timely applications of fungicides are important. Depending on the rainfall pattern in an area, two to three sprays are usually applied at intervals of 30 to 60 days, starting when the fruit size is approximately 25mm in diameter (KALLIDEEN 2020). Copper fungicides are very effective. The fungicide flutriafol is also registered in the MAPA (Ministry of Agriculture, Livestock and Food Supply) for the control of the disease in Brazil (AGROFIT 2023). Rodríguez *et al.* (2020) indicate the use of DMI fungicides (demethylation inhibitors) and the mixture of DMI

and QoI (quinone outside inhibitors), and their rotation with CuOCl during the fruit susceptibility period, in order to reduce the resistance induction risk in *P. purpurea* to these unisite fungicides (DMI and QoI).

Biological control with *Bacillus subtilis* through field sprays integrated with copper oxychloride has been reported in South Africa (KORSTEN *et al.* 1997). Insect control, root rot management and good orchard hygiene, which includes the removal of infected avocado prunings, are all beneficial (DANN *et al.* 2013).

Avocado varieties show great variation on susceptibility to disease. Collinson, Fuchsia and Pollock are considered less susceptible; Linda, Prince and Simmonds are moderately susceptible and, Fuerte, Lorena, Ryan, Sharwil and Wagner are highly susceptible (PEGG & COATES 1993; PICCININ *et al.* 2016; KALLIDEEN 2020; RODRÍGUEZ *et al.* 2020).

## ANTHRACNOSE

Anthracnose occurs in all countries where avocados are grown, including countries with a Mediterranean climate and low rainfall (SILVA *et al.* 2022). It is the most serious postharvest disease of avocado in high rainfall growing regions, which can cause significant losses during storage and transport of fruits (PICCININ *et al.* 2016). The disease can also cause significant preharvest losses of fruit in some cultivars (FITZELL 1987).

### Symptoms

Anthracnose mainly affects fruits, with symptoms manifesting after harvest and during ripening, although symptoms can develop before harvest on unripe fruit. Symptoms that develop during ripening initially appear as small, light-brown circular lesions. As lesions enlarge, they become slightly sunken in the centre and dark-brown or black, reaching part of the fruit pulp or necrotizing it completely (DANN *et al.* 2013). Under humid conditions, salmon or orange coloured spore masses may be produced in the centre of lesions (Figure 4A). External symptoms of anthracnose are difficult to see on ripe 'Hass' fruit due to the dark skin colour.

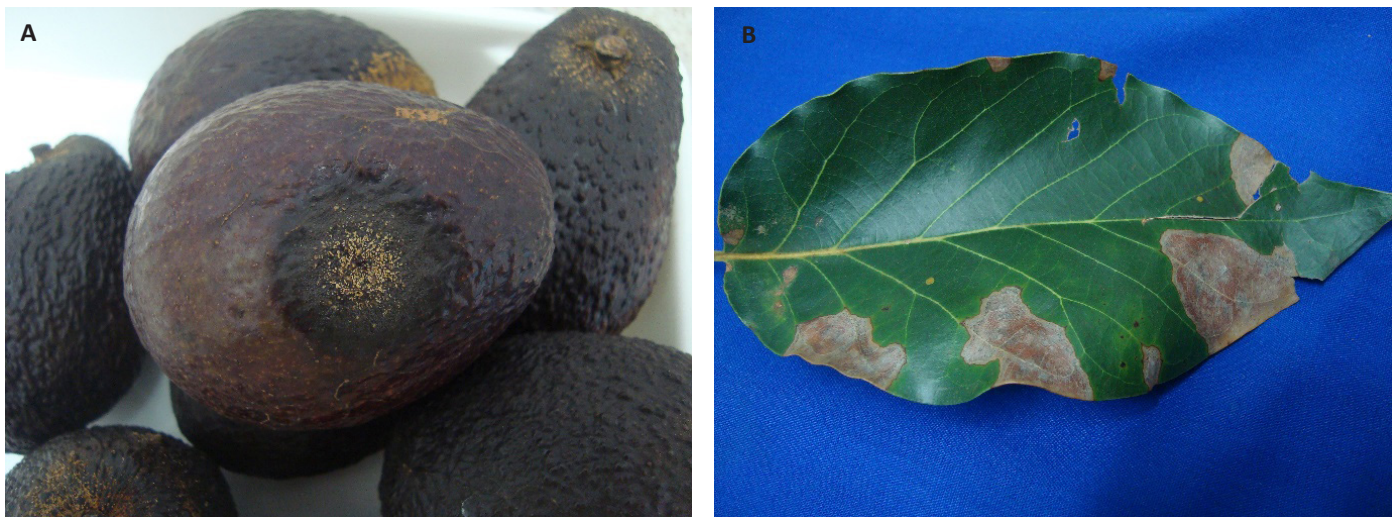
Fitzell (1987) described two types of lesions that occur on unripe fruit prior to harvest. Type 1 lesions are similar to those described above for ripe

fruit, typically occur singly, and are usually associated with some form of skin injury (e.g. insect damage), which appear mainly on the north (sunnier) side of the canopy. About 90% of the fruit that develop Type 1 lesions abscise (drop) before harvest. Type 2 lesions are small (1–5 mm in diameter) and often associated with lenticels and high inoculum level in the field. Only a small percentage of Type 2 lesions are associated with skin injuries. Approximately 35% of fruit which develop Type 2 lesions fall before harvest. Both Type 1 and Type 2 lesions are common on fruit of 'Fuerte', while in the Hass variety type 2 anthracnose is more common (SILVA *et al.* 2022).

Another pre-harvest disease caused by *C. gloeosporioides* (Penz.) Penz. & Sacc. *sensu lato*, pepper spot, occurs on avocado fruit in Australia (WILLINGHAM *et al.*, 2000) and South Africa (SCHOEMAN & MANICOM 2002). Numerous minute, shiny black spots (0.1–0.5 mm in diameter) develop on the surface of the fruit, particularly on the warm northern side of trees, and sometimes form 'tear stain' marks on the fruit surface. The pedicel and sometimes leaf petioles show the same spotting. Eventually pedicels may be covered with a shiny black scab. Symptoms in South Africa and Australia generally appear from mid- to late summer onwards and spots are particularly prevalent on 'Hass' fruit affected by mild sunburn (WILLINGHAM *et al.* 2000). Pepper spot symptoms have also been observed on 'Fuerte' fruit in Australia and on 'Pinkerton' fruit in South Africa (SCHOEMAN & MANICOM 2002). *Colletotrichum gloeosporioides* from pepper spot caused more severe symptoms than anthracnose isolates on 'Hass' fruit inoculated in the field and on leaf petioles of young 'Hass' trees inoculated in the glasshouse (GIBLIN *et al.* 2010).

Anthracnose symptoms rarely develop on avocado leaves, flowers, and branches, except under very humid conditions (PEGG & COATES 1993). Symptoms on the leaves are characterized by necrotic spots of dark color, with well-defined edges and irregular shape, occupying the margins of the leaves or the spaces between the veins (Figure 4B), which under severe attack can cause defoliation (SILVA *et al.* 2022). Infection on flowers can cause drought, floral abscission, premature drop and/or rotting of young fruits. On the branches and shoots, there are dark and dry necrosis (PICCININ *et al.* 2016).





**Figure 4.** Symptoms of anthracnose in avocado fruit (A) and leaf (B).

### Causal agents

Before the use of multiple gene phylogeny for the identification of *Colletotrichum* spp., *C. gloeosporioides sensu lato* was the most common species found in association with avocado anthracnose, followed by *C. acutatum* J.H. Simmonds *sensu lato* (DANN *et al.* 2013). According GIBLIN *et al.* (2010), pepper spot is caused by *Colletotrichum gloeosporioides sensu lato*. Pepper spot isolates caused more severe symptoms than anthracnose isolates on ‘Hass’ fruit inoculated in the field and on leaf petioles of young ‘Hass’ trees inoculated in the glasshouse (GIBLIN *et al.* 2010). Host-association and morphological characterization was previously used to identify *Colletotrichum* species; but due to the overlapping morphological characters, a polyphasic approach is now recommended for accurate species identification within this genus (CAI *et al.* 2009; CAI *et al.* 2011; LIU *et al.* 2016). In the last decade, with the application of molecular-based phylogenetic analysis using multiple markers was established that these two species belong to two complexes called *acutatum* and *gloeosporioides*, with several other species included within them. At least 21 species of *Colletotrichum* have been identified causing anthracnose in avocados worldwide, belonging to the *C. gloeosporioides*, *C. acutatum*, *C. boninense*, *C. gigasporum*, *C. magnum*, and *C. dematium* complexes (HUNUPOLAGAMA *et al.* 2015; SHARMA *et al.* 2017; GIBLIN *et al.* 2018; FUENTES-ARAGÓN *et al.* 2020; SOARES *et al.* 2020; HOFER *et al.* 2021; BUSTAMANTE *et al.* 2022). Species in a species complex are closely related, and have similar behavior of host infection and colonization (DAMM *et al.* 2012a; DAMM *et al.* 2012b; WEIR *et al.* 2012).

In southeastern Brazil, *C. siamense* Prihast., L. Cai & K.D. Hyde was the main specie associated with avocado anthracnose, and *C. karsti* You L. Yang, Zuo Y. Liu, K.D. Hyde & L. Cai was also found less frequently, considered as part of the *C. gloeosporioides* and *C. boninense* Moriwaki, Toy. Sato & Tsukib. species complex, respectively (SOARES *et al.* 2020). In Mexico, 11 species were the causal pathogens of avocado anthracnose, however, the most prevalent species was *C. siamense* and the most widespread was *C. karsti* (FUENTES-ARAGÓN *et al.* 2020).

The fungus produces conidia on acervuli, which are saucer-shaped fruiting bodies. Short, simple and colourless conidiophores produce conidia in abundance. Long, brown setae may or may not be produced among conidiophores. These setae are approximately 4-8 x 200 µm, brown, slightly swollen at the base and tapered at the apex. Conidia form on hyaline to lightly brown conidiophores in acervuli which are irregular and roughly 500 µm in diameter. Conidia are unicellular and colourless when viewed alone, but may also appear pink, orange or salmon coloured. Colony colours range from cream to gray, pink, salmon or dark gray (DANN *et al.* 2013). Although the shape of the conidia is not considered a uniform feature to characterize the *Colletotrichum* complexes (DAMM *et al.* 2012a; DAMM *et al.* 2012b), conidial shape allowed to distinguish strains among the three species complexes found in avocados in Mexico; the *gloeosporioides* complex had straight and rounded ends, while the *boninense* complex had a prominent basal scar and the conidia of the *acutatum* complex had at least one acute end (FUENTES-ARAGÓN *et al.* 2020). Conidial size cannot delimitate species or complexes and ranged

from 13.4–17.0 × 3.8–5.0 µm on PDA. Appressoria showed variability in size and shape, but they also could not delimitate species or complexes.

The teleomorph of *C. gloeosporioides sensu lato*, *Glomerella cingulata* (Stonem.) Spauld and Schrenk, can sometimes be observed in culture (SIMMONDS 1965), and as well as the teleomorph of *C. acutatum sensu lato*, *Glomerella acutata* Guerber & J.C. Correll (GUERBER & CORRELL 2001), probably plays a minor role in the epidemiology of the disease.

### Epidemiology

Conidia of *C. gloeosporioides sensu lato* are produced in large numbers on dead leaves and twigs entangled in the avocado tree canopy (FITZELL 1987). Infected fruit still hanging on the tree can also be an important source of inoculum. In Israel, *Colletotrichum* sp. was confirmed to survive during the winter in green leaves and branches in the orchard (SHARMA *et al.* 2017).

During rainy weather, conidia are washed down through the canopy of the tree. Infection mainly occurs during extended periods of warm showery weather, and fruit are susceptible at all stages from fruit set to harvest (PETERSON 1978; COATES *et al.* 1993a). In the study of the temporal progress of avocado anthracnose, *Colletotrichum* sp. was detected in 60-86% of open flowers (FISCHER *et al.* 2019). In the presence of free water, most conidia deposited on the fruit surface will germinate within 7 h (PARBERY 1981). Each germinated conidium produces a germ tube that attains a length of 10–20 mm. Approximately 5–6 h after germ tube emergence, development of a terminal appressorium commences. Initially the apex of the germ tube becomes swollen, enlarging back towards the conidium. Upon reaching maximum size, the wall of the appressorium thickens and darkens considerably, and a central germ-pore develops in the ventral wall (PARBERY 1981). An infection peg emerges from this germ-pore and penetrates the outer wax layer and cuticle of the fruit skin. Growth of the infection peg is arrested in the cuticular region, where it remains quiescent until fruit ripening (PRUSKY *et al.* 1990; COATES *et al.* 1993b). It is thought that the fungus is unable to colonize unripe tissue due to the presence of antifungal compounds (dienes) in the peel of the fruit (PRUSKY *et al.* 1983). During ripening, diene levels decline in the peel, allowing fun-

gal growth to resume. Underlying cells of the peel and the flesh are colonized, leading to the development of symptoms. In advanced stages of lesion development, acervuli are produced beneath the fruit surface. Eventually the cuticle and epidermal cell walls are ruptured, and conidia are released in a mucilaginous matrix. Conidia are then dispersed by water and wind (FITZELL & PEAK 1984). The spatial distribution of anthracnose in Mexico avocado orchards presented aggregation and localized sources of infection (TAPIA-RODRÍGUES *et al.* 2020). In a Brazilian study evaluating the disease within the trees, random dispersion was observed, indicating that their initial inoculum is evenly distributed in the plants (FISCHER *et al.* 2019).

When Type 1 lesions develop before harvest in cultivars such as ‘Fuerte’, insector mechanically induced wounds are thought to cause localized ripening, thereby allowing the fungus to colonize tissue that would normally be resistant. In contrast, Type 2 lesions develop without any skin damage (FITZELL 1987). Insect injuries occurring on branches, leaves, inflorescences, peduncles, and young fruits may be associated with symptoms of anthracnose (REIS *et al.* 2018).

Temperature and leaf wetness are important factors in the development of anthracnose, as is the case for pepper spot. In South Africa, there was little development of pepper spot at temperatures below 18°C irrespective of leaf wetness. At temperatures above 18°C, however, levels of disease increased with increasing temperatures and canopy wetness; a combination of temperatures over 18°C and rainfall exceeding 20 mm for a 10–14 day period was associated with severe pepper spot development (SCHOEMAN & MANICOM 2001).

### Management

Both pre-harvest and postharvest strategies are required for anthracnose control in avocado. Copper-based fungicides are usually used to control anthracnose, typically 2 to 3 applications during fruiting (FISCHER *et al.* 2018). In Australia and New Zealand, copper sprays are applied on a calendar basis at intervals of 14–28 days from fruit set to harvest (WILLINGHAM *et al.* 2001; EVERETT *et al.* 2007). Copper spray programs recommended for control of cercospora spot and scab are also used for anthracnose control. In orchards where copper is not controlling the disease, the use of fungicides

with trans-laminar activity or systemic fungicides from flowering is also recommended. Currently registered for the control of the disease in Brazil, in addition to cuprics (copper oxychloride, copper hydroxide and copper oxide), there are the fungicides mancozeb, thiophanate-methyl, thiabendazole, azoxystrobin and difenoconazole (AGROFIT 2023). To mitigate the development of strains of the pathogen resistant to unisite fungicides, limiting the numbers of applications during a season and using these fungicides in association with standard copper spray programs are typically employed. Anthracnose control in 'Hass' avocado was obtained with three sprays of azoxystrobin, alternating with two to three applications of copper oxychloride during fruiting (FISCHER *et al.* 2018).

Orchard hygiene is also an important strategy in the control of anthracnose. Removal of infected fruit and dead wood and leaves entangled in the tree canopy can help reduce inoculum levels (SHARMA *et al.* 2017) and should be performed at least annually. Ventilation within the canopy can be improved through tree pruning, thus making conditions less favourable for the build-up of inoculum. Controlling insect pests such as fruitspotting bugs and fruit flies can reduce the incidence of Type 1 anthracnose. Maintaining the fruits stem-end at the time of harvest helps to prevent the disease from occurring on the stem-end, as well as injuries to the fruit should be avoided during harvest and post-harvest operations (PICCININ *et al.* 2016). Ideally, best avocado harvesting and packing practice should recognize that unripe fruit must not experience drop heights of 30 cm or higher, as a way of not favoring the occurrence of body rot, mainly caused by *Colletotrichum* spp. (PERKINS *et al.* 2020).

Postharvest application of fungicides is an important strategy in the overall management of anthracnose, particularly in areas of high disease pressure. Tiabendazole has been shown to be an effective fungicide for postharvest anthracnose control in avocado (FISCHER *et al.* 2018), however, due to increased restrictions on the use of postharvest fungicides, mainly in avocado importing countries, most packinghouses no longer use fungicides in fruit processing in Brazil.

Controlled ripening and postharvest temperature management can have a major influence on anthracnose development in harvested fruit. There is a strong correlation between fruit ripening time

and anthracnose development in avocado (DARVAS 1985; HOPKIRK *et al.* 1994), and treatments that reduce ripening time (e.g. ethylene treatment) also tend to reduce anthracnose. Fitzell & Muirhead (1983) found that temperatures over 24°C increased anthracnose levels significantly in 'Fuerte' avocado fruit, and recommended that fruit be ripened at 17°C. Hopkirk *et al.* (1994) recommended that 'Hass' avocado fruit be cool-stored at 6°C and then ripened at 15°C. However, if fruit were to be ripened at 25°C, final fruit quality was better if fruit were not cool-stored prior to ripening.

Although the disease occurs in all avocado varieties, it is recommended to avoid planting varieties that are highly susceptible to the disease, such as Fuerte, Nabal, Rincon and Wurtz, and that are harvested in periods of rain (DANN *et al.* 2013).

With continuing pressure to reduce pesticide use, interest in alternative disease management strategies has increased. In Australia, studies indicated that fruit from 'Hass' avocado trees grafted to 'Velvick' rootstock had lower levels of anthracnose than those from trees grafted to 'Duke 6' and 'Duke 7' rootstocks (WILLINGHAM *et al.* 2001; MARQUES *et al.* 2003). In addition, reductions in anthracnose were correlated with lower nitrogen and higher calcium levels in leaves and fruit skin (WILLINGHAM *et al.* 2006), and balanced fertilization should be adopted, avoiding excess nitrogen and adopting the correct calcium management, mainly through liming. To reduce rots of 'Hass' avocados in New Zealand, it is recommended that growers apply calcium to increase the calcium + magnesium/potassium ratio above 0.065 in fruit (EVERETT *et al.* 2007).

Tree crop load has also been shown to have an impact on fruit anthracnose levels, with 'Hass' fruit from high yielding trees shown to have lower disease levels and lower nitrogen:calcium ratios than low yielding trees (HOFMAN *et al.* 2002; DANN *et al.* 2013).

There are several reports of the efficiency of methods considered alternative in the control of anthracnose under experimental conditions with naturally infected avocados. Postharvest thyme oil fumigation (96 µL L<sup>-1</sup>) for 24 h had positive effects on enhancing anthracnose disease resistance during storage and also gave a residual effect during the simulated shelf life of avocados (BILL *et al.* 2016). The incidence of anthracnose disease was also reduced in 'Hass' avocado fruit treated with meth-



yl jasmonate and methyl salicylate vapours at 100  $\mu\text{mol L}^{-1}$  for 24 h (GLOWACZ *et al.* 2017). Postharvest spraying of lemon grass essential oil (*Cymbopogon citratus* (DC.) Stapf) (1 mL  $\text{L}^{-1}$ ) and immersion of the fruits for 1 min in suspension with sodium bicarbonate (20 g  $\text{L}^{-1}$ ) (FISCHER *et al.* 2018) or 1.5% w/v chitosan for 3 min (OBIANOM *et al.* 2019) also reduced the incidence of anthracnose in avocados. According Obianom *et al.* (2019), anthracnose control by chitosan was a combination of its antifungal and eliciting properties.

### BRANCH CANKER AND STEM-END ROT

Avocado branch canker and dieback is a fungal disease that occurs more intensely during periods of higher humidity and temperature in the orchard. Dense and poorly managed orchards, especially in phytosanitary control, tend to be more affected. Although there are no reports on yield losses due to avocado branch canker in Brazil, this disease has the potential to infect many avocado trees, resulting in considerable yield reductions. Stem-end rot is common in most avocado-producing areas, but is generally less important than anthracnose.

#### Symptoms

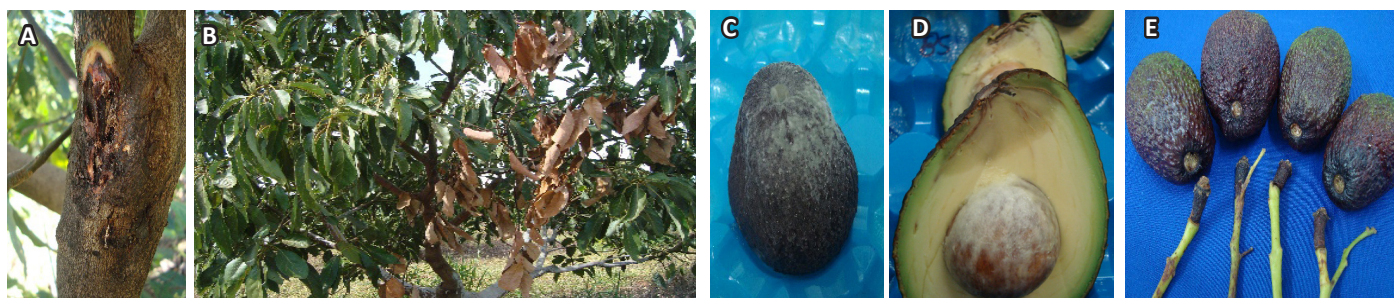
Symptoms include cankers on the trunk, branches and twigs that cause dark, friable bark, often with the dried brown-white exudate of the C<sub>7</sub> sugar, perseitol. Underneath the canker, the bark and wood turns red-brown or brown and can penetrate into the heartwood (Figure 5A), sometimes with a characteristic wedge-shaped discoloration visible on cross section, interrupting the normal flow of sap and causing the drying of branches and shoots, and the leaves may not fall, remaining on the plants (Figure 5B). In severe attacks, the disease can even cause the death of the plant. Even if the plant does not die, the impairment of the branches affects the accumulation and viability of the reserves necessary

for fruiting, which are located mainly in the trunk and branches (CHANDERBALI *et al.* 2013), which can reduce the productivity of the orchard (VALENCIA *et al.* 2019). The disease can be a serious problem in new plantings that are established with nursery stock that is latently infected at the graft union. Where infection kills the graft union, the dead scion retains a dry brown canopy, while shoots and green leaves sprout up from the rootstock. The graft union may be unusually swollen and rough before the young tree dies. Cutting inside at the graft union reveals dark, discolored wood that can extend through the entire width of the small trunk (MENGE & PLOETZ 2003).

On fruits, as the name implies, rotting starts at the stem-end from where it progresses throughout the fruit, being more common in post-harvest. For most types of stem-end rot, external symptoms appear as a dark-brown to black rot with well-defined margins. Mycelial growth is sometimes seen on the surface of lesions, particularly during advanced stages of symptom development and under humid storage conditions. With the exception of stem-end rot caused by *Colletotrichum* spp., vascular tissue becomes discoloured, typically in advance of flesh decay symptoms (Figures 5C and D) (DANN *et al.* 2013). Premature fruit drop can also occur, as pathogens can infect the peduncle region (Figure 5E) (PICCININ *et al.* 2016).

#### Causal agents

Branch canker and stem-end rot are also caused by the same pathogens, belonging mainly to the Botryosphaeriaceae family and less frequently to the Diaporthaceae family. The teleomorphs of these pathogens occur rarely as small pustules (irregular-shaped pseudothecia) on dead bark (MENGE & PLOETZ 2003). Black, knob-like pycnidia are commonly observed on old diseased branches (MENGE & PLOETZ 2003; MCDONALD *et al.* 2009) and fruits.



**Figure 5.** Symptoms of branch canker (A), branch blight (B), stem-end rot (C and D) and stem-end necrosis in avocado (E), causing fruit drop.

The identification of the Botryosphaeriaceae genus can be obtained by scraping the surface of the mummified fruit with a scalpel blade and observing the white cirri with conidia released from the pycnidia.

Several species of the Botryosphaeriaceae family (*Diplodia mutila* (Fr.) Mont., *D. pseudoseriata* C.A. Pérez, Blanchette, Slippers & M.J. Wingf., *D. seriata*, *Dothiorella iberica* A.J.L. Phillips, J. Luque & A. Alves, *Fusicoccum aesculi*, *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., *L. pseudotheobromae* A.J.L. Phillips, A. Alves & Crous, *Neofusicoccum austral* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, *N. luteum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, *N. mangiferae* (Syd. & P. Syd.) Crous, Slippers & A.J.L. Phillips, *N. mediterraneum* Crous, M.J. Wingf. & A.J.L. Phillips, *N. nonquaesitum* Inderb., Trouillas, R.M. Bostock & Michailides, *N. parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips and an unknown species of *Neofusicoccum* sp.) have already been identified causing symptoms of branch canker / dieback and stem-end rot in orchards in several countries, such as Taiwan (NI *et al.* 2009), United States (MCDONALD & ESKALEN 2011), Thailand (TRAKUNYINGCHAROEN *et al.* 2015), Brazil (FIRMINO *et al.* 2016), Chile (VALENCIA *et al.* 2019) and Spain (ARJONA-GIRONA *et al.* 2019). *L. theobromae* was the most frequent (73%) in avocado seedlings with graft failure, in a nursery in Egypt (MONIR *et al.* 2021). Other fungal genera may occasionally be associated with branch canker or stem-end rot symptoms, such as *Phomopsis perseae* Zerova, *Thyronectria pseudotrichia* (Schwein.) Seeler, *Fusarium* spp., *Pestalotiopsis versicolor* (Speg.) Steyaert, *Neopestalotiopsis* spp., *Bipolaris setariae* (Sawada) Shoemaker, *Neocosmospora perseae* Sand.-Den. & Guarnaccia and *Rhizopus stolonifer* (Ehrenb.: Fr) Viull. (DARVAS & KOTZÉ 1979; JOHNSON & KOTZE, 1994; GUARNACCIA *et al.* 2021; FIORENZA *et al.* 2022).

Differences in aggressiveness between genera and species of pathogens have already been observed in inoculated avocado seedlings, with a decreasing level of virulence for *N. parvum*, *N. luteum*, *N. mediterraneum*, *N. australe*, *C. gloeosporioides* and *L. theobromae* (ARJONA-GIRONA *et al.* 2019). In Italy, *N. parvum* was also the most aggressive species on seedlings and avocado fruit in relation to *Diaporthe foeniculacea* and *D. sterilis* L. Lombard, Polizzi & Crous (GUARNACCIA *et al.* 2016). In another study, *N. nonquaesitum*, *N. parvum* and *Diplodia*

*pseudoseriata* were the most aggressive in inoculated seedlings, while *L. theobromae*, *N. australe* and *N. parvum* were the most aggressive in fruits (VALENCIA *et al.* 2019).

The identification of Botryosphaeriaceae species is only possible with the phylogenetic analysis of genic regions, since there is an overlap of morphological characters between the species (PHILLIPS *et al.* 2013; VALENCIA *et al.* 2019). Phylogenetic characterization of Botryosphaeriaceae species is possible by combining information about the large subunit of the nuclear ribosomal RNA gene (LSU-rpb2), the internal transcribed spacer 1 and 2, including the intervening 5.8S nrDNA gene (ITS), translation elongation factor 1-alpha (tef1) and the  $\beta$ -tubulin gene (tub2) (YANG *et al.* 2017).

*Fusicoccum aesculi* produces fluffy grey mycelium with discrete pycnidia on PDA or stromatic multilocular fruiting bodies on oatmeal agar (OA). Conidia are fusiform to navicular, 12–25  $\times$  4–6  $\mu$ m, hyaline and single-celled. The teleomorph is occasionally produced on OA and has been found in litter beneath avocado trees. On twigs, pseudothecia are subglobose to pyriform, 201  $\times$  120  $\mu$ m, and immersed beneath the epidermis. On OA, ascostromata are hemi-lenticular and up to 10 mm wide. Asci are eight-spored, bitunicate and irregularly biseri-ate. Ascospores are hyaline, single-celled, fusiform and 16–25  $\times$  4.5–9.5  $\mu$ m (MENGE & PLOETZ 2003). Colonies of *N. parvum* grown on PDA are initially grey-white, becoming grey to greenish grey on the upper surface with dense, fluffy, aerial mycelium, and greenish grey on the reverse side. Conidia are hyaline, ellipsoidal with a rounded apex and a flat base, measuring 17.1-19.6  $\times$  4-6.9  $\mu$ m (GUARNACCIA *et al.* 2016). Abundant pycnidia and conidia develop when isolates are cultured on 2% water agar sterilized pine needles as substratum at 25°C under near-UV light for 2 weeks.

Colonies of *L. theobromae* are grey to black, fluffy, with abundant aerial mycelium and a fuscous grey to black reverse. Pycnidia are simple or compound, often aggregated, stromatic, ostiolate, frequently setose and up to 5 mm in diameter. Conidiophores are hyaline, simple, sometimes septate, rarely branched, cylindrical and arising from the inner layers of cells lining the pycnidial cavity. Conidiogenous cells are hyaline, simple, cylindrical to subobpyriform, holoblastic and annelidic. Conidia are initially aseptate, hyaline, granulate, subvoid

to ellipsoid-oblong, thick-walled and base truncate. Mature conidia are one-septate, cinnamon to fawn, often longitudinally striate and 20–30 × 10–15 µm. Paraphyses when present are hyaline, cylindrical, sometimes septate and up to 50 µm long. On host tissue, pycnidia are immersed, later becoming erumpent, simple or grouped, 2–4 mm in diameter, ostiolate and frequently pilose with conidia extruding in a black mass. A teleomorph, *Botryosphaeria rhodina* (Cooke) Arx, has been described, but ascospores play a minor role in infection (DANN *et al.* 2013).

*P. perseae* produces pycnidia subepidermally that gradually become erumpent and black. Pycnidia are 400–500 × 200–225 µm, containing one, occasionally two or three cavities, first yellow or indistinct, tapering, with a round pore at top. The upper peridium is thick, but the lower one thinner. Conidiophores are indistinct. Conidia measure 7–10 × 2.3–2.5 µm, are fusiform and contain lipid fragments at the two poles (DANN *et al.* 2013).

### Epidemiology

Wounding is a prerequisite for infection on twigs and branches; thus, cankers are associated with pruning wounds, sunburn, frost damage, mechanical damage, bark split from wind damage, and grafting wounds. The incidence and severity of these cankers is greatest in trees subjected to drought stress, nutrient deficiencies, waterlogging, temperature extremes, or damage by insects or other pathogens (DANN *et al.* 2013).

Many of the stem-end rot pathogens, such as the anamorphs of *Botryosphaeria*, *P. perseae* and *C. gloeosporioides sensu lato*, occur as ‘endophytes’ or ‘phelloglyphs’ in the extra-cambial tissue of avocado twigs, branches and pedicels (JOHNSON & KOTZE 1994; HARTILL & EVERETT 2002). Although this colonization is not pathogenic, it may ultimately lead to the colonization of inflorescence and fruit stem-end tissue by these fungi, as has been reported for mango (JOHNSON *et al.* 1992). The relative importance of this mode of infection in the development of stem-end rot is unclear, as a high proportion of stem-end rot infections can be initiated during harvest (HARTILL & EVERETT 2002). However, it is believed that the endophytic occurrence can become pathogenic when the host undergoes some type of stress (DANN *et al.* 2013; SLIPPERS & WINGFIELD 2007; SLIPPERS *et al.* 2017) or in post-harvest. The patho-

gens *Lasiodiplodia* and *Fusicoccum/Neofusicoccum* were found in early-developing fruits, 0.8 cm long, after disinfestation of the fruits with sodium hypochlorite (5 g/L<sup>-1</sup>) for five minutes and treatment with paraquat (3 g/L<sup>-1</sup>) for a minute (FISCHER *et al.* 2019). Infection in fruits also occurs by lenticels under high humidity conditions, being favored by injuries to the fruit skin (NAVARRO *et al.* 2022).

Pathogens survive in the orchard on crop residues due to their saprophytic capacity. Spores are produced mainly under high humidity conditions on dead leaves, fruits, twigs and branches and are spread by splashing rain, wind or by swaying branches during fruit harvest (HARTILL & EVERETT 2002; ESKALEN *et al.* 2013). Most infections of the stem-end remain quiescent until fruit ripening commences. Pathogenicity and hierarchical analysis of the genetic variation indicated that *N. luteum* isolates from diseased branches and fruits present inspecificity by plant organ and could not be genetically separated, indicating that diseased branches can serve as a source of inoculum for infections in the fruits and vice versa (TWIZEYIMANA *et al.* 2013b).

### Management

It is recommended to use healthy seedlings and to sanitize the tools for the removal of branches used in grafting. In the orchard, disease severity can be reduced by addressing stress factors, particularly drought stress and maintaining good general tree vigor. Pruning and harvesting operations must be carried out under dry environmental conditions. Dead leaves, wood and fruits within the canopy should be pruned and removed from the orchard. It is expected that high density plantings which require repeated pruning will increase disease incidence. Field sprays of such plantings with copper fungicides may prove beneficial, particularly after pruning operations or protecting girdling and phosphonate injecting wounds with copper paste may help prevent invasion by canker-causing pathogens (DANN *et al.* 2013; PICCININ *et al.* 2016). Mesostemic and systemic fungicides recommended for anthracnose control also show some efficacy in controlling the disease (RADWAN & HASSAN 2016; MONIR *et al.* 2021). The fungicides boscalide+pyraclostrobin (MONIR *et al.*, 2021), carbendazim (RADWAN & HASSAN 2016), azoxystrobin+propiconazole and metconazole (TWIZEYIMANA *et al.* 2013a), although without registration for avocado crop in Brazil (AGROFIT 2023), also



preventively controlled the disease in seedlings or branches inoculated with the pathogens. The monitoring and control of coleborer populations aim to reduce the infection by the pathogens in the branches.

Reduction in the incidence of stem rot was associated with calcium applications, aiming to increase the ratio calcium + magnesium/potassium above 0.065 in the fruit (EVERETT *et al.* 2007). Fruit should be picked at optimum maturity levels, as immature fruit readily develop stem-end rot, and clippers used for harvesting should be disinfested regularly (HARTILL & EVERETT 2002). Postharvest temperature management, as described for Anthracnose, is another important strategy.

Varieties from the Mexican race were more resistant to the disease than the Guatemalan ones in visual observations on the trunk of the plants in the orchard. However, in plants inoculated with *N. ribis* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, it was shown that the pathogen can affect both varieties, requiring, according to the authors, further studies to be able to conclude about the behavior of avocado varieties (HALMA & ZENTMYER 1953). In a survey of the severity of branch canker / dieback and incidence of stem-end rot caused by *L. theobromae*, in avocado orchards in Egypt, the Hass and Fuerte varieties were the most susceptible, followed by Eitinger, Duke and Reed, while the varieties Bekon and Benkerton recorded the lowest percentage of infection (Radwan & Hassan, 2016). Greater susceptibility of the Hass variety in relation to Bacon was also observed for stem rot, caused by *D. foeniculacea* (GUARNACCIA *et al.* 2016).

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