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Essay

Botryosphaeriaceae in avocado crops

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Abstract

The objective of the literature review was to know the epidemiological and identification status of species that have been carried out with respect to members of the family Botryosphaeriaceae of agricultural importance. The paper was prepared in 2023 based on scientific research reports in the field of phytopathology, of epidemiological reports of the disease known as canker and its characteristic symptom of dieback. The disease is distributed throughout the avocado-producing region and reported in several crops and is widespread throughout the world and there is a coincidence that members of Botryosphaeriaceae are severe and virulent phytopathogens that generate great economic and biological losses in the case of native species. In Mexico, in avocado crops, this group of phytopathogens was reported in the 1980s (Coria, 1985), with favorable conditions for the disease to develop, it colonizes the root and base of the crown, invades vascular bundles, and takes nutrients for its development; the disease is triggered by a stress condition and is influenced by temperature, precipitation, moisture, pH, and soil texture. In the field, the biological effectiveness of molecules for their control may be a feasible alternative, but preventive and cultural practices are more effective. Given the phytosanitary situation involved in avocado production, it is necessary to implement integrated management alternatives, but not before knowing the associated phytopathogens, this with molecular techniques due to the genetic plasticity and morphological similarity they present.

Keywords:

canker, control, disease, sequence, taxonomy.

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The family Botryosphaeriaceae is first described in the 1820s, with the genus *Sphaeria* as a representative member (Crous *et al*., 2006). The genus *Botryosphaeria* as such was established until 1863 and was described as most of the Ascomycetes, with characteristic structures called ascomata, which are spherical-shaped, and tunicate and bitunicate asci; they were identified as saprophytes because their fruiting occurred only on dead tissue of woody plants (Bega *et al*., 1978; Brown and Britton, 1986; Von Arx, 1987; Michailides, 1991; Swart and Wingfield, 1991).

So far, Botryosphaeriales are made up of six families: Aplosporellaceae, Botryosphaeriaceae, Melanopsaceae, Phyllostictaceae, Planistromellaceae and Saccharataceae (Phillips *et al*., 2019; Zhang *et al*., 2021). Most Botryosphaeriales taxa are reported as endophytes, that is, they are found within the host in healthy plant tissues for indefinite periods of time according to the phenological cycle and plant host species (Slippers *et al*., 2007).

Important species within Botryosphaeria, such as *Diplodia*, *Dothiorella*, *Lasiodiplodia*, *Neofusicoccum*, *Phyllosticta*, *Pseudofusicoccum* and *Saccharata*, include well-characterized endophytes in different crops (Slippers and Winfield, 2007; Crous *et al*., 2008; Crous *et al*., 2016; Zhang *et al*., 2021). There are detailed studies that sampled the great diversity of these fungi, they are also included within a range of plants, species with different lifestyles such as saprobes and obligate phytopathogens, but no less important (Slippers and Wingfield, 2007).

Physiology of Botryosphaeria

The ascospores and conidia of Botryosphaeriales have a diverse morphology, they may be pigmented or hyaline, septate or aseptate and with the presence or absence of a mucilaginous sheath; the conidia can be fusiform to ovoid or elliptical. Hyaline and aseptate conidia become one or two septate and some species develop brown coloration before germination, such as *Diplodia corticola*, *D. cupressi* and *D. mutila*, with conidia very similar to each other because they belong to the same genus (Phillips *et al*., 2013). Thick-walled, hyaline or brown, diplodia-like conidia can also be found in the family Botryosphaeriaceae.

Finally, the innermost cells are thin-walled and hyaline. The pycnidium ostiole is simple, circular, and central. The conidiogenous cells are hyaline in appearance and are located perpendicular to the walls of the pycnidium, thus orienting themselves towards the center of the cavity. The melanin produced by these structures can be found in phytopathogenic species as well as in taxa with saprobic habits and this contributed significantly to survival when environmental conditions are adverse (Belozerskaya *et al*., 2017). This means that melanin production is more related to phytopathogenic fungi because it is directly related to virulence and severity (Nosanchuk and Casadevall, 2003; Belozerskaya *et al*., 2017).

Cycle of the disease of the family Botryosphaeriaceae

The life cycle of most phytopathogenic fungi of the family Botryosphaeriaceae (Figure 1), begins when conidia or ascospores come into contact with the host through natural openings or openings artificially made by mechanical damage. Natural openings can be lenticels (these are small structures found on stems, roots, and branches that allow gas exchange) (Everett *et al*., 2008) or even in panicles in cases of ‘branch rot or dieback’. Openings artificially created by mechanical damage can be made accidentally by pruning, grafting, harvesting, planting, or other cultural work, as well as by hailstorms, fires, or insects (Everett *et al*., 1999).

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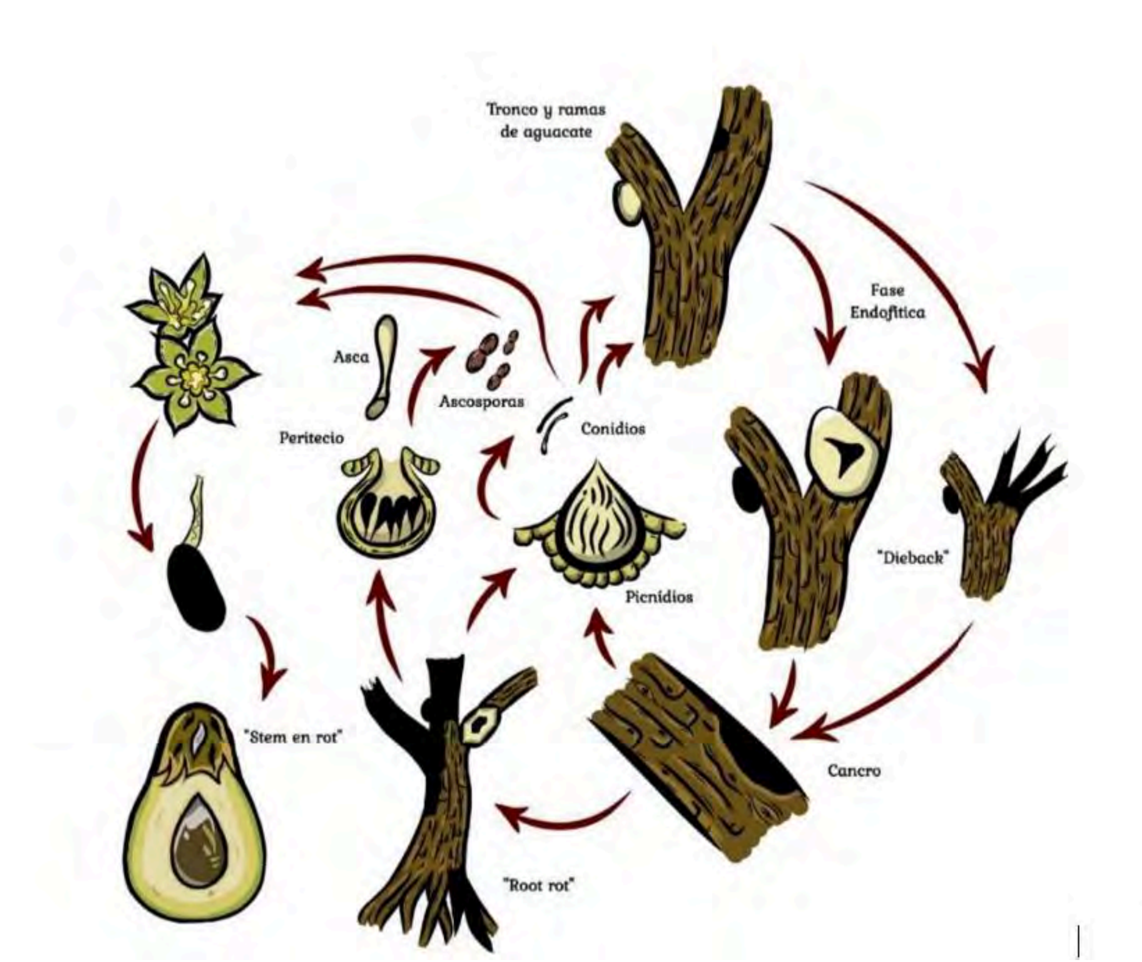
Figure 1. Pathogenesis cycle of fungi of the family Botryosphaeriaceae that cause cankers in avocado trees.



The pathogenesis cycle of these fungi begins when conidia or ascospores infect the plant through wounds or lenticels; the fungus produces enzymes that attack the cambium and sapwood, degrading them to use them as nutrients. Once the plant tissues are colonized, the production of conidia begins in the pycnidia (perithecia: spore-forming structures that emerge through the infected bark). These conidia are responsible for the appearance of the secondary cycles of the disease in other organs of the plant.

In the tissues that are already dead, together with the production of conidia, sexual structures are generated, which, once mature, produce infective ascospores that give rise to new primary infections. The fungus survives on cankers and infected branches and shoots, as well as in dead leaves in the soil.

The infection begins with the germination of conidia or ascospores, which make the infection advance when they feed on the host, this can also occur due to the influence of stress (Coakley *et al*., 1999). The disease cycle can develop simultaneously and produce symptoms in different parts of the plant, this is very serious and the disease can be even worse if the host is under biotic pressure from possible agents that cause phytosanitary problems, such as the presence of pests or diseases, such as the occurrence of root rot caused by *Phytophthora cinnamomi*, a common disease in avocado crops (Desprez-Loustan *et al*., 2006).

The fungus produces enzymes that degrade the host's tissues, representing a source of nutrients and energy for the phytopathogen. After tissue colonization, the production of conidia begins in

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pycnidia, asexual reproductive structures that emerge through the bark of branches and trunk (Valencia, 2019).

Conidia are responsible for secondary infections of the disease, they are infectious structures that spread very easily through rain splashing and wind, through contaminated insects and tools, sporulation is favored by high moisture conditions (Pérez *et al*., 2017).

Diagnosis

Cankers on woody tissue are the most common and consistent symptom caused by genera of the family Botryosphaeriaceae; it is an alteration expanded to the cortical tissues of the central part of the tree, produces necrosis and death of the tissue, this symptom is expressed with aqueous or gummy exudates depending on the parasitized species and with or without hyperplastic reactions observable in the surrounding living cells. The diagnosis of this disease is fundamentally based on the identification of the syndrome along with the specific signs produced by the fungus according to the species (fruiting bodies) (Sánchez *et al*., 2013; Zlatkovic *et al*., 2016).

Symptoms in woody tissue can also range from small superficial areas of bark that penetrate between the cambium to the sapwood to elongated lesions that can occupy a considerable area on the wood from the base of the trunk to aerial areas of the tree. The lesions usually appear mainly on the trunk, are associated with wounds made during the weakening and advance of the phytopathogen and are observed as extensive areas of infected bark with a notable change in their typical coloration, a white to cream exudate resulting from the expulsion of sap from the tree as a hypersensitivity response to the wound, which later crystallizes and remains in the form of a whitish looking powder, these are bordered by elongated bark bulges, about 5 to 20 cm in length or more. If the lesions coalesce, they can girdle and kill the tree. Black pustules appear on the dead cortex of the lesions, which correspond to the pseudothecia of the phytopathogen (Doll *et al*., 2015; Lawrence *et al*., 2015).

Botryosphaeriales as an important phytosanitary problem in woody species

Some pathogenic genera of Botryosphaeriales species have a peculiar and at the same time very virulent behavior, this is called ‘inactive pathogens’, it is a habit that is also known as endophyte, they can coexist within the plant and develop in an asymptomatic way (such as the genus *Botryosphaeria* and *Lasiodiplodia* species) and have the ability to cause disease after a trigger, such as drought or plant weakening caused by another infection by another, weaker phytopathogen (Slippers and Winfield, 2007; Prusky *et al*., 2013; Wyka and Broders, 2016).

Among the most notable and agriculturally important hosts are pome fruit trees such as pomes, seed fruit trees, nut trees and berries (Slippers *et al*., 2007), there are also reports of epidemics in native plants of different habitat types (Marincowitz *et al*., 2008; Pavlic *et al*., 2008; Jami *et al*., 2015); in contrast, Botryosphaeriales also have genera that possess narrower but equally severe hosts on infected plants, for example, *Diplodia sapinea* in *Pinus* sp. species and even some very host-specific ones, for example *Eutiarosporella darliae*, *E. pseudodarliae*, and *E. tritici-australis* in wheat (Thynne *et al*., 2019).

Botryosphaeriales associated with avocado crops

In the commercial avocado orchards located in the producing region of the state of Michoacán, there is an area made up of municipalities that stand out for different agroclimatic conditions and that belong to one of the sites with a considerable cultivated area for several decades (Téliz *et al*., 1999).

There are reports of some members of the family Botryosphaeriaceae and although they are already classic, they are reported as agents that cause infections and that are associated with the symptom of canker in the trunks and branches, which is generally located at the base of the trunks, although it

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can also affect the roots near the base of the stem (Coria, 1985). The disease is found in all avocado-producing areas of Michoacán, there is no orchard with at least one plant with this symptom since in young orchards, it is very common to find it also at the graft junction (Jiménez, 1987).

Campos (1984) suggests an incidence of 5 to 20%, but at present, a higher value is estimated due to poor agricultural practices and the spread of the disease due to increasingly adverse climatic conditions. As Coria (1985) has reported, since the 1980s, there is an incidence of canker in trees of up to 29% in orchards located in Ziracuaretiro, Michoacán. The canker of the trunk, *Nectria galligena* has been reported with a further set of fungi, this due to work carried out by Martínez (1974); Jiménez (1987) in the state of Michoacán.

Economic importance of Botryosphaeriaceae canker in avocado crops

Given the increase in incidence in avocado orchards and the economic impact of the symptoms of the disease generated by members of this family and recent reports of the emergence of new species of Botryosphaeriaceae in tropical plants, it is possible that several species of this family may be associated with cankers and death of *Persea americana* Miller plants in Mexico. For effective management of the disease, it is necessary to have a clear understanding of the etiology of the disease, both morphological and molecular identification are essential to determine the distribution of species and the epidemiology of their disease (Slippers and Wingfield, 2007; Mehl *et al*., 2013).

Importance of molecular identification of Botryosphaeriaceae species

As in all identifications at the genus and species level, the family Botryosphaeriaceae is no exception and it has been carried out according to the morphological characteristics of anamorphs, such as conidiomata and conidia mainly (Denman *et al*., 2000). Nevertheless, it is a practice in which it had not been considered that these fungi biologically possess genetic plasticity, which can phenotypically modify their morphology and it can be activated by climatic factors and even by the host where they develop, and on the other hand, many of the species of Botryosphaeriaceae do not easily produce pycnidia or conidia in synthetic culture media (Liu *et al*., 2012).

A rapid and feasible detection method is to perform PCR (polymerase chain reaction) tests based on DNA sequence analysis, this would provide an efficient way to detect and identify plant pathogens (Martin *et al*., 2000; Abdollahzadeh and Zolfaghari, 2014). In their research, White *et al*. (1990) suggest the use of the ITS (internal transcribed spacer) region of ribosomal DNA (rDNA), it is ideal to use it as a primer for a PCR-based detection, ITS regions are generally conserved regions within a variable species, other gene regions commonly used to infer phylogenetic relationships and elucidate species within the family Botryosphaeriaceae are: tef1-#, tub2 and rpb2, which allowed species to be identified easily and with a high degree of certainty (Michailides, 2002; Ridgway *et al*., 2011; Ni *et al*., 2012; Cruywagen *et al*., 2016).

Disease control

Control measures for cankers caused by species of the family Botryosphaeriaceae should be preventive since once lesions are present, curative measures are ineffective (Junta de Andalucía, 2010). It has been proven that vigorous, healthy plants with good agronomic and nutritional management are more resistant to this type of disease and although there are optimal conditions for an infection to develop, such as stress, weakening, excessive irrigation, shading and sometimes mechanical damage, it will prevent the pathogen from establishing the disease (Ceja *et al*., 2000).

In general, it is recommended to keep plantations under favorable conditions in order to avoid any type of unnecessary stress (Junta de Andalucía, 2010). An essential practice within the cultural management that has been very effective for good control is the removal of the affected branches

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during the dry periods of summer or those of winter rest, always before the first rains of spring, since with these, the greatest dispersion of the spores occurs and thus we can reduce the spread and proliferation of the disease.

These preventive measures include better control with integrated management, by ensuring that the environmental humidity is as low as possible (recurrent pruning), avoiding mechanical wounds in the bark of the tree, disinfesting tools, and sealing pruning wounds. Another complementary type of preventive control is with fungicide products, in directed sprays via drench to the root system, trunk and branches, with products with systemic biological activity, active ingredients such as Benomyl, a broad-spectrum systemic fungicide capable of being absorbed by woody tissue, thus inhibiting fungal infection (Luque *et al*., 2008; Junta de Andalucía, 2010).

However, some molecules of this type have phytosanitary restrictions that make them no longer usable. In this way, alternatives to these fungal molecules have been sought and in their search, contact protectors have been implemented, such as coppers (non-absorbable) or mixtures of both (systemic and contact) (Luque *et al*., 2008).

Conclusions

The family Botryosphaeriaceae is a group of fungi with members of important phytophages worldwide, the epidemiology of Botryosphaeriales is the causative agent of many diseases such as cankers, dieback, and root rot, distributed in avocado producing areas. The incidence of the disease has increased considerably and it has become a limitation in the production and development of the crop. Due to the above and under the demand generated by this crop, it is necessary to be able to quickly and truthfully identify the species associated with the disease in order to know their biology and thus design control alternatives.

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