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## Management of soil borne fungi on coffee

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#### Abstract

Soil borne fungi can be very importan on coffee plantations and in the nursery. One of the key measure to get vigorous plants in the field is the quality of the seedling and the site of planting. The great majority of the diseases on seedlings came from the nursery. Another source of pathogens is to plant in soil that has suffered clearing of forests. The soil's organic matter contains soil pathogens that attack coffee seedlings after planting. Sometimes plants can die. The main soil borne disease of coffee are: Rhizoctoniosis, Rosellinia root rot, Ceratocystis canker stain and Coffee Wilt Disease. For these reasons the main measures of integrated control is exclusion of the pathogen and avoidance.

Keywords: management, soil borne fungi, coffee

## Introduction

Rhizoctoniosis is a serious disease in nurseries and seedlings, causing damping-off in pre and post emergence. The disease is caused by the fungus *Rhizoctonia solani*. Seedlings in the field, in a permanent planting area, can be attacked about a year after planting, which determines a great delay or death in relation to the normal development of the healthy plant. The disease is of great economic importance, causing damage to coffee seedlings, planting failures or weak seedling production. The disease can attack coffee plants in the field for up to a year after planting, especially when the coffee plantation is done in areas of devastated forest (Zambolim *et al.* 1997; 1999; 2000)<sup>[53]</sup>.

## **Symptoms**

The attack, when in pre-emergence, causes the seedlings to die before they reach the soil surface; however, the most common infection occurs in post-emergence. The stem is attacked in the neck region, where brown lesions are noted, which can reach 1 to 3 cm in length. The disease tends to rotate the stem, in which the necrotic region is observed to be strangulated due to rot of the bark, due to the penetration of the mycelium into the tissues. As a consequence, there is an interruption of the sap circulation, which causes the aerial part of the seedling to wilt and, later, its death. When the lesion reaches the wood, it can cause the stem to break in the attacked region, causing the seedling to tip over. The pathogen obtains sugars and pectin for its growth, by means of pectinolytic enzymes. Consequently, tissue death and maceration occur as the hyphae advance into the host's tissues. The fungus cannot attack cellulose and lignin, so fibrous plants remain upright despite being dead. Under conditions of high humidity, the mycelium of the fungus develops brown or gray color. The fungus attack the seedlings on the lower part of the stem, where a light brown, sometimes reddish constriction occurs, which distorts and affects the functioning of the vascular bundles, which prevents the arrival of water on the cotyledonal leaves and the first true leaves (Gaitán, 2003) <sup>[26]</sup>. The tipical symptoms of the disease is in the Figure 1. The disease manifests itself up to a year after planting. The lesion appears on or just above the soil surface. The stricken region, which is characterized by strangulation, covers 1 to 2cm or more of the stem. At the upper limit of the lesion, scar tissue is formed, which covers an appreciable area of dead wood. The swelling comes from the accumulation of descending sap. It is believed that this injury is caused by the infection that occurred late in the nursery (Gaitán, 2003)<sup>[26]</sup>.

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Photo: G. Hoyos, Cenicafé.

Fig 1: Symptoms of damping-off caused by Rhizoctonia solani in coffee seedlings before and after the germination of the seeds

## Etiology

Damping-off or ''The Mal del Talluelo'' is a very heterogeneous complex of a group of fungi, where the *Rhizoctonia solani* Kühn fungus stands out. The pathogen is very important in the production of coffee seedlings and sometimes in the field conditions in plants till one year. It is a fungus that inhabits the soil, with great saprophytic capacity, and can live in cultural debris from one year to the next, in the form of sclerotia (Mora, 1992) <sup>[39]</sup>. In *C. canephora* there is another species described as the causative agent of rhizoctoniosis, *Rhizoctonia bataticola*, which causes the same symptoms.

The assexual classification of the fungus is (Gaitán et al., 2015) <sup>[27]</sup>: Kingdom - Fungi; Phylum - Basidiomycota; Class -Hyphomycetes; Subclass incertais Order sedis; Agonomycetales; Family - Agonomycetaceae; Genera -Rhizoctonia; Genera and Espécie - R. solani. The sexual phase the fungus is classified in the Kingdom - Fungi; Phylum -Basidiomycota; Class - Agaricomycetes; Subclass Incertae sedis; Order - Cantharellales; Family - Ceratobasidiaceae; Genera - Thanatephorus; Genera and Species - Thanatephorus cucumeris (A. B. Frank) Donk, 1956. The Rhizoctonia solani fungus is divided into three groups according to the number of nuclei per cell: (i) the genus Rhizoctonia is multinucleated with three or more nuclei per cell with wide hyphae of 6 to 10 µm and the telemorph is Thanatephorus cucumeris Donk., which produces basidia with four apical sterigmas where basidiospores that are very labile ovoid form and hvalines: (ii) Binucleate Rhizoctonia constitutes the other group whose sexual status is Ceratobasidium Rogers and the hyphae have an average wide size of 4 to 7 µm; (iii) The last group presents the perfect state of Waitea Warcoup and Talbot and they have multinucleated mycelium and include R. oryzae and R zeae, of great relevance in world food security crops.

## Epidemiology

The fungus is an inhabitant of the soil and survives from one season to another in the soil by means of sclerotia, as mycelium colonizing crop residues or as a saprophyte (Galindo *et al.*, 1983) <sup>[28]</sup>. Rain is the main agent in the dissemination of sclerotia or mycelial fragments from the soil to the first leaves of the plant. The pathogen needs a film of water to cause infection, and when the leaves dry, the growth of the mycelium is stopped. The incubation period at 18°C to 24°C, with a range that oscillates from 16°C to 30°C is 36 to 72 hours. Soil very humid and poorly drained soil favor the development of the disease (Mora, 1992) <sup>[39]</sup>.

Nursery seedlings and plants in the field are favored by excess

moisture, high nitrogen content and shade. The attack is severe during spring and summer, due to the abundance of rain and optimal temperatures between 25 ° C and 28 ° C. Deep planting favors the faster manifestation of the pathogen, especially when the pit is filled prematurely. Sowing seeds treated with fungicides in washed sand without organic matter does not provide a favorable environment for the development of the fungus.

The disease appears at sowing and in nursery when contaminated substrate with the pathogen are used. Soils from old coffee plantations and forests rich in organic matter usually contain the fungus inoculum. Infection of seedlings in the growth phase occurs by hyphae or by the germination of sclerotia that colonize the hypocotyl región (Flentje *et al.*, 1963; Dodman *et al.*, 1968) <sup>[22, 17]</sup>. High humidity (above 90%) and temperatures of 18 ° C to 28 ° C are favorable to the attack of the pathogen in sowing and nursery conditions. In these conditions, the inoculum of the fungus spread when sprinkler irrigations are made. Soils contaminated with the fungus are also responsible for the spread of the pathogen to the nurseries. Forest soil used to prepare nurseries for coffee seedlings to growth is the main source of contamination.

The light affects the initiation of the perfect state of the fungus, which grows and develops under conditions of continuous indirect or intermittent light. This condition favors the formation and release of basidia and basiodiospores; dark conditions do not favor the formation of basida. The fungus need a film of water, permanent dew and little light to infect the plants.

## **Disease management**

There is no resistant varieties of coffee to the disease. To control the disease must be preventive using cultural practices (Zambolim et al., 1997; 1999; Solano & Brenes, 2012; Coedero, 2013) <sup>[53, 48, 15]</sup>. The main measures are: (i) Avoid terraces originating from forest or 'capoeira' rich in organic matter to form seedlings and sand rich in organic matter to form seedlings in nurseries and sowing; (ii) Organic compounds, when used in the formation of the seedling substrate, must be well decomposed and free of the pathogen; (iii) It is recomended to use artificial substrate with appropriate fertilization because it is free of pathogens; (iv) Spray the substrates used for seed germination and seedling growth with appropriate fungicides; (v) Treat the seeds with protectant and / or systemic fungicides before sowing; (vi) As another option to formation the seedlings use soil originating from the B horizon without organic matter, mix it with washed sand in a 3: 1 ratio and fertilizers; (vii) Fill the plastic bags, which must have the size of at least 22.0 x 11.0 cm, to form seedlings; (viii) Spray the substrate before to growth coffee seedlings with the appropriate fungicides; (ix) In contaminated nurseries eliminate not only the bags of seedlings with symptoms, but also those around the diseased seedlings and spray the rest with appropriate fungicides; (x). During seedling growth phase, spray with specific fungicides against *R. solani*. (xi) Choose the appropriate place to install the nursery, avoiding humid, poorly drained and shaded places and control irrigation and shading of the nursery. To form coffee seedlings it is recomended to use artificial substrates because it is free of pathogen.

#### Rosellinia root rot Introduction

Rosellinia root rot has been observed for a long time in most coffee regions in the world. The disease is caused by Rosellinia bunodes (Berk. & Br.) Sacc. The disease is of great concern for the coffee grower as it is difficult to eradicate when installed in the fields. It usually occurs in new coffee plantations, planted in recently cleared land, appearing in plant ridges. The plants dies from a center where there are decaying tree trunks and remains of old forest, especially in tropical regions. In addition to the coffee tree, numerous cultivated plants are attacked by the disease (cacao citrus, avocado, rubber tree inga, macadamia, yam, cassava, banana, plantain, forest trees, serveral species of noncomercial trees, fruit trees and any type of vegetable that allows their survival. There are no data on the damage that the disease causes to coffee growing. The disease is restricted to rich in decomposing organic matter. Today it is difficult to find this disease in the field because it is prohibited to deforest to form coffee crops or any other agricultural activity.

The fungus is a soil inhabitant in the forest soils. The disease was diagnosed in the roots of the coffee by Berkeley and Broome in plantations in Ceylan now Sri Lanka, in the year 1870. Some time later, Zimmerman identified and verified the pathogenic relationship between *Rosellinia bunodes* Berk. & Br. (Sacc.) and samples obtained from roots of coffee trees, obtained on the island of Java, in the year 1902.

The pathogen that attacks coffee is *Roselinia bunodes* Berk. & Br. (Sacc.) causing black root rot in Central America (Costa Rica, Honduras, Guatemala and El Salvador) and also India, Brazil and Colombia (Caicedo, 2003)<sup>[5]</sup>; *R. pepo* (Fernandez & Lopes, 1964)<sup>[20]</sup> causing star-shaped rot in South America; and *R. arcuata* Petch. the causal agent of black root in India (Kannan, 1995).

In the coffee zone of Colombia, the presence of these pathogens dates back to the 30s and their incidence increased as coffee crops were established in forest areas on soils with abundant organic matter, former cocoa crops and in a cassava production system. Losses of up to 50% of trees are recorded due to R. *bunodes* attack when a coffee plantation is established on abundant cassava roots generating focus that is difficult to manage (Castro, 1999). All varieties of coffee are susceptible to the disease.

## Symptoms

The external symptoms are similar to those caused by *Ceratocystis fimbriata* Ellis. & Halst. The coffee plants affected are located in foci. The first symptoms in the aerial part appear in coffee trees with three to five years old are yellowing, withering and falling of the leaves, death of the branches and death of the coffee plants. The primary site of infection is at the root collar área, where the bark is superficially cracked and mass of mycelium can be found as black dots or short lines embedded

in the internal wood when caused by R. bunodes or as a white stellate mycelium growth under the bark of affected roots when caused by R. pepo (Gaitán et al., 2015) [27]. There may be abundant flowering, but some of the flowers may fall before fertilization. The coffee cherries are small and malformed, some remaining green and others the seed do not form. In a short time, the leaves fall off, leaving the bare branches, which dry quickly, with the death of the coffee tree. Safe diagnosis of the disease requires examination of the root system. The roots located immediately below the surface of the soil show the typical symptoms of blackening and disorganized bark. On the surface of the roots, whitish and branched filaments are observed, which are the rhizomorphs of the pathogen (Figure 1). Subsequently, the rhizomorphs become dark, presenting a series of nodes along their branches. Under the bark, thick black layers are formed, which appear as streaks when the bark is cracked. From the lower surface of this layer, black branches leave and invade the wood. Making transversal cuts in the roots, these branches appear as black punctuations and, through longitudinal cuts, black lines are observed. The death of the plants occurs in focus (Fernández & López, 1964)<sup>[20]</sup>.



https://www.google.com/search?q=Imagen+deLlaga+estrellada+en+el+cultivo+de+cafeto+causada+por+Rosellinia+pepo+en+cafeto&r

Fig 1: Symtoms of white rot caused by *Rosellinia pepo* in coffee plantations.

## Etiology

The genera Rosellinia was originally described por De Notaris in 1844, on the surface of wood in decomposition. Rosellinia belongs to the class Ascomycetes, subclass Hemiacomycetes, Order Sphaeriales and family Xylariaceae. The especies identified in coffee roots in Colombia was is R. bunodes (Berk. & Br.) Sacc. and R. pepo Pat. (Lópes, 1965; Gaitán, 2015) [27]. The fungi have two forms the ascosporic (teleomorphic) Rosellinia spp.; and the conidial (anamorph) Dematophora sp., which is rarely found in nature (Bermudez & Carranza, 1992)<sup>[3]</sup>. Asci are hyaline, cylindrical, 9 -12 x 250 - 350 micrometers, long stalked, and unitunicate with eight ascospores. Ascospores are elipsoidal, without celular appendages. Several dark reddish to brown synemata arise from a common enlarded base, are 1.5 mm x 40.0 - 140.0 micrometers long, and are composed of septate hyphae with 3 - 4 micrometers in diameter. Conidiophores are formed on the top of synnema, up to 70 micrometers long frequently di- or trichotomously branched, and light brown. Conidia are one celled, simple ellipsoidal or ovoid, hyaline to pale brown, and smooth. The R. bunodes conidia are  $3.0 - 4.0 \ge 6.0 - 7.0$  micrometers and the *R. pepo* conidia are 2.0 - 3.0 x 5.0 - 9.0 micrometers (Gaitán et al., 2015)<sup>[27]</sup>.

The genus Dematophora form with white mycelium, with dilations next to the septa in the old hyphae. Rosellinia is still characterized by the production of rhizomorphs on the surface of the attacked organ. Rhizomorphic cords are forms of resistance.

## Epidemiolgy

Both species R. bunodes and R. pepo are soilborne, facultative saprophytes that become pathogenic on dead stumps or log on newly cleared land and spread nearby living trees, thereby affecting a circle of trees around the infection center by root contact (López & Fernández, 1966) <sup>[36]</sup>. The pathogen start to colonize with a thin, brilhiant white mycelium that superficially invades the larger roots, changing progressively until it turns gray. R. bunodes mycelium then form black masses on the wood and black stripes and dots under the bark; R. pepo produce dense gray, cottony mycelium superficially over the root and fan- or star-shaped mycelium under the bark. After the substrate have been consumed, synnemata emerge from the root or on the infested soil. Rosellinia spp. can survive saprophytically for several years on rotted roots in the soil. Infested soil and disease root debris may be transported by farm operations. Cassava debris left on the soil is a rich médium for fungal growth. R. bunodes and R. pepo are related to residues of shade trees, especially Inga spp. The residue of this plant specie become sources of initial infection from where they advance by contact between roots and generate foci of several affected trees (Castro, 1999). Optimum natural conditions for development of Rosellinia spp. include soils rich in organic matter, temperaturas of 20 - 28 °C, soil moisture of 70 - 80%, and pH of 4.0 - 7.0. Extremely well-drained or flooded soils have strong inhibitory effects on pathogen growth. Inoculated seedlings develop the disease after 9 days on seedlings and 20 days on plantlets, when the pathogen reaches the root collar. The mycelium initially develops on the root surface and then penetrates the cells until reaching conducting vessels and the medulla. Black mycelia colonize the conducting vessels, forming the black stripes and dots in the roots that characterize the disease (Gaitán et al., 2015) [27]. Rosellinia species are native to virgin forests surviving on roots, logs, rotten stumps and other plant residues, in a shady and humid environment, which can become pathogenic. They are natural forest dwellers. After the trees are felled, rhizomorphs develop on the decomposing stumps and roots. Under favorable conditions, the fungus extends its rhizomorphs in the soil, until it reaches the roots of the coffee trees planted in the newly cleared lands. The penetration of the rhizomorphs of the pathogen in healthy plants usually takes place in the roots located close to the surface of the soil. In order for penetration to occur, injuries are essential. Rhizomorphs grow on and under the bark; its black branches invade the wood, where they emit secondary branches in different directions, which penetrate inside the cells. This distribution results in the destruction of protective tissues and conductive vessels, with symptoms appearing in the aerial part and leading the plant to death. The roots die and, in a humid and shady environment, conidia and periteciums can appear successively. Few plants die in the first year after planting. Usually coffee trees die in rows, which gradually widen as the fungus reaches other plants. High temperatures, high precipitation and little sunstroke favor the development of the fungus. Excess of humidity do not favor fungus growth (Fernández & López, 1964) [36]. Directly solar radiation and very high temperatura inhibit the growth of the pathogen (Castro & Esquivel, 1991)<sup>[14]</sup>.

The *R. pepo* infection occurs in a similar way to *R. bunodes*. The pathogen condenses its mycelium at various points along the

roots, forming gray-white fans or stars that turn dark as they age (Realpe, 2001). Both fungus species can be disseminated by the root contact, soil transport, shoes. Cacao, cassava, trees of the forest, fruit trees, platano, pineple and tomato are hosts of the two pathogens (Caicedo, 2003)<sup>[5]</sup>. The disease occurs in foci in the field.

## **Disease management**

Resistance in coffee varieties is unknown. The management of the disase must be preventive. Plants with symptoms and signals of the disease must be removed from the field. Care should be taken to avoid spread inoculum (contaminated soil and roots) in the growth area. So all affected trees and their neighbors should be uprooted and destroyed, and care should be taken to prevent movement of remaining fragments of diseased roots in the soil. Foci of affected trees must be isolated by trenching, and immediate replanting must be avoid. Wait one ou more year to plant coffee seedlings in the contaminated field (Castro, 1999). The soil affected to the pathogen should be exposed to the solar radiation of at least six months to reduce the amount of inoculum of the pathogen from the soil (Aranzazu *et al.*, 1999)<sup>[1]</sup>.

Soil form forest should not be usesd to form seedlings to avoid inoculation of the pathogen; one should also avoid these soils for filling pits in the field; remove stumps, roots and tree trunks after logging. To form coffee seedlings it is recomended to use artificial substrates because it is free of pathogen.

## Ceratocystis canker stain

## Introduction

Canker stain is caused by the complex Ceratocystis fimbriata sensu lato, is one of the most important disease of coffee. The disease was first reported in 1900 from Indonesia, where it ws discovered on the Island of Java. The disease subsequentely appeared in Colombia in 1932 (Caicedo, 2003; Gaitan et al., 2015)<sup>[5]</sup>. Canker stain is now found in various part other parts of Central and South America, as well as in Indian coffee-growing áreas. The disease is widely distributed worldwide, being present in Ecuador, Colombia, Guyana, Peru, Costa Rica, Mexico, Guatemala, the Dominican Republic, Trinidad, Jamaica, Haiti, the Philippines, Sri Lanka, Fidji and New Guinea. In Brazil and West Africa it affects other crop species such as citros, cacao, mango, forrest trees (Castro, 1999). The disease was diagnosticated in Colombia since 1930 (Caicedo, 2003) <sup>[5]</sup>. Malaguti (1956) refers to the death of more than a million cacao trees of the valuable Criollos in the central valleys of Aragua in Venezuela. Since 1960, the fungus has been reported on various hosts, especially in the cocoa areas of the state of Sucre, where the death of nearly a million plants also occurred. Capriles de Reves reported the disease in various cocoa areas of Venezuela in 1972. The disease in not exclusive to coffee trees, but is of utmost economic importance in cultivation. The presence of the fungus is very common in various roots and tubers, such as yam and sweet potato where it generates very considerable losses. The yield losses caused by the disease are of relevant economic importance, mainly in regions where adequate conditions for its development are present. The fungus can cause heavy losses in adult, poorly nourished plantations and especially in poorly drained soils. It can reduce the number of plants per aera of about 20 to 50% on the renewed coffee plantations on the montaineus áreas of the South America (Caicedo, 2003)<sup>[5]</sup>. All the varieties of coffee are susceptible to the disease (Castaño, 1953a; Castro, 1999).

## **Symptoms**

The fungus causes canker stain and wilt diseases, mainly in fruit, forest, and ornamental trees. It affects plants of any growing age. The first apparent symptom of canker stain is the yellowing of the mature leaves, which present dark green areas between the ribs, later the leaves wilt, take on a reddish, uniform coloration, slightly curl on their vertical axis, remain hanging and dry in one to two months. After wilt, an accelerated death occurs throughout the plant. Foliar wilt appear three months to a year and then generalized to the whole coffee plantation. Diseased plant dies in a period of a few weeks (Fgure 1A, B and C).

The structures of the fungus are located in vascular roots, therefore, the disease is considered vascular. The affected plant emits tylosae that obstruct the xylem-carrying vessels and imposes a supply of water to the various tiles of the plant, causing the water deficiency.



Source: Barba *et al.*, 1961<sup>[2]</sup>.

A

В

С

Fig 1A: Internal symptoms that resemble decorative figures of dark coffee color to light; 1B. External symptoms in plant height and 1C. Symptoms of total wilting and dried branches affected by the fungus.

The primary site of infection, as seen by vascular streaking, is the root collar or other parts of the stem where dark sunken lesions can bark. When the bark is removed, darkly colored lesions with a steaked pattern can be found in the infected wood which can also extend into the roots. In advanced stages of the disease, lesions girdle the trunks, resulting in tree death. Common sites for infection are the pruning wounds created when plants are refuvenated by stem stumping, then new shoots become infected wilt and die.

In the branches, stem and superficial roots there are dried, and depressed areas that shows perforations caused by insects (Figure 2), which are ambrosial beetles (Coleoptera -Curculionidae - Scolytinae). Ambrosia beetles are common insects present in the bult log of injured, weakened, dead or recently felled trees. Losses have not been quantified, their economic importante is well recognized. Ambrosia beetles attack weakened, dying, and recently cut or killed trees. They can attack freshly cut lumber and lumber in decks before it is dried, and they can cause pinhole defects and dark staining in the outer wood. Galleries are formed in the sapwood or heartwood and damage the wood. Because ambrosia beetles tunnel into the wood, they are considered wood borers rather than bark beetles. Adults introduce ambrosia fungi that stain the wood, and lower its value. Ambrosia beetles feed on the fungus rather than the wood. The most obvious sign of an ambrosia beetle attack is the fine, white boring dust that accumulates at the base of the tree and in the bark crevices. Adults bore straight into the tree, creating perfectly round, small-diameter holes. If

the bark is removed, the entrance points of adult ambrosia beetles and galleries are distinctive and are often surrounded by a dark brown or black fungal stain.

Ambrosia beetles are small, roughly 2 mm long, elongated and cylindrical insects that can be found in branches, trunks and superficial roots. These beetles have a forced symbiosis with the fungi, which grow in galleries or tunnels that they themselves drill into the xylem of the tree that hosts them. In their larval stage, beetles feed on fungi until they reach adulthood and emerge carrying symbiote fungi in structures called micangia Carraro - Moreira, 1997).

Perforations are detected by sawdust and gummy exudates accumulated at the foot or trunks of plants (Figure 2). On the bark, multiple bluish-brown spots are observed that grow in a cortical and medullary direction, intersected by galleries, where the different stages of the insects of the Taxa Scolytidae develop in the different stages of development. In Florida, the red bay leaf ambrosia beetle (Xyleborus glabratus) is the main vector of the fungus (Raffaelea lauricola) that causes the disease called laurel wilt. In coffee cultivation, the insect is Xyloborus sp. This insect disseminate the fungus C. fimbriata through the galleries (spots) where the mycelium of the fungus is found in the asexual and sexual phase. These spots can appear from the base of the trunk to the area of the fork and branches, also when discovering the root area, necrotic areas with perforations of the insect can be observed. No apparent symptoms are observed in the fruits, although the leaves detach from the side and remain hanging.



Image: http://trec.ifas.ufl.edu/tropicalentomology/factsheets/ambrosia\_beetles\_es.shtml IFAS-UF. Perforations Ambrosia beetle (Xyleborus)

Fig 2: Perforations performed by the insect *Xylosandrus (Xyleborus) morigerus* (Bland) in avocado plant, from where the galleries and the development of sexual and asexual structures of the fungus are produced.

## Etiology

The genus Ceratocystis was described for the first time in 1890, and its economic importance was pointed out during the first decades of the XX century. *Ophiostoma ulmi* the sexual stage caused severe epidemy on *Ulmus* spp. in Europe and in the United States (Caicedo, 2003) <sup>[5]</sup>. The genus was proposed by Ellis and Halsted in 1890. The asexual stage (anamorph) of Ceratocystis is Chalara and Thielaviopsis, and Ophiostoma to the genus Graphium.

The fungus Ceratocystis spp. belongs to the class of ascomycetes that represent some of the most plant pathogens causing canker stain and wilt diseases specially in fruit, forest and ornamental trees. Structures of the sexual states of Ceratocystis are perithecia with globose bases and diameter of 138 - 185 micrometers, giving rise to long necks of 408 - 627 micrometers typically terminating in distinct ostiolar hyphae. Asci are evanescent and seldom seen in the case of Ceratocystis fimbriata Ellis & Halst. Ascospores are hat shaped, 2.4 - 2.7 x 5.7 - 6.5 micrometers, and borne in sticky masses at the apices of the ascomatal necks (Upadhyay, H.P 1981). The asexual state of these fungi is in the genus Thielaviopsis, typified by distinct tubular conidiophores and rectangular endoconidia that are 2.4 x 19.4 micrometers and produced in chains. These fungi also produce distinct, dark colored chlamydospores that facilitate survival in the soil.

The causal agent of canker stain of coffee is broadly referred to as the sexual stage (teleomorph) *C. fimbriata sensu lato*. This specie form resistance structure clamidospores between the afected tissues (Kile, 1993). Contemporary studies on *C. fimbriata* have shown that this fungus represents a complex of cryptic species that have been described during the last decade. Recent studies on isolates of the pathogen in Colombia have shown that the isolates reside in two disctinct phylogenetic clades other than *C. fimbriata sensu lato*. Isolates in these clades have been given the names *C. colombiana* M. van Wyk & M.J.Wingf., sp. nov. and *C. papillata* M. van Wyk & M.J. Wingf., sp. nov. It is not known whether the same Ceratocystis species in the *C. fimbriata sensu lato* species complex cause canker stain of coffee elsewhere in the world (Gaitan *et al.*, 2015).

## Epidemiology

The fungus C. fimbriata is considered as facultative saprofyte,

present in all type of soils and altitudes from 800 to 2000 m. The fungus can live in the soil in dormant stage and debris as macroconidia (Castaño, 1953a). Soil represents one of the major source of inoculum for the Ceratocystis spp. causing canker stain of coffee. These fungi can easily be isolated from the soil taken from infected lands by baiting with freshly cut coffee twigs (Gaitan *et al.*, 2015).

The dissemination is by the wind, water, human beeings, work tools, and the movement of the soil. It penetrates in the plant tissue by wounds during the cultural practices (Castro, 1999). Infected coffee trees are usually randomly scattered in the fields, without any clear pattern of distribution. Cultural practices seems to help in the distribution of the fungus in the plantation.

The disease is classified as a monocyclic, with a spread pattern that is typically to the monomolecular type epidemiological curve. *Ceratocystis* spp. in the *C. fimbriata sensu lato* species complex have close associations with causal insects such as picnic beetles.and flies that feed onwounded plant tissue. The insects of the Scolytidae family are the natural disseminators of the disease. In their life cycle, the beetles excavate deep galleries in the wood, usually reaching the heartwood. Their association with ambrosia fungi is a dependent mutuallism making of this group of beetles the most highly evolved within the order. *Ceratocystis* spp. produce fruity odors that are attractive to these insects, and when the fungi are present on the surface of the lesions, their spores attach to the insect that carries them to fresh wounds.

Damage caused by ambrosia beetles can vary greatly among places. In some areas, aggressive control programs are required to reduce economic damage to wood products. The symbiosis is made possible due to special ectodermal structures that preserve and nourish the spores during the transportation to new hosts. Upon feeding a suitable host, the ambrosia beetles excavate a complex gallery system. The fungi sprout and grow in the gallery walls producing nutritious spores eaten by adults and larvae. The insecta need ergosterol and nitrogen present in the diet for oviposition, development, and reproduction. The excretIon of Uric Acid by the insect is used by the fungl.

Dissemination over short or long distances will depend largely on the frequency and intensity of the various cultural practices, the population of disseminating insects and the climatic conditions of the region. Acording to Gaitan *et al.*, (2015), there have been no detailed studies or the insect associations with The propagules of *C. fimbriata* are disseminated by strong winds, floods and irrigation. In addition to the movement of materials, agricultural equipment, as well as animals and workers.

Canker stain is reported in all coffee growing regions of the world, with some exceptions. The fungus has the ability to to attack multiple hosts and also the ability to survive as a saprophyte in the Rain Forest Biome, where the conditions for coffee growing create an environment conducive to perpetuate itself indefinitely.

The fungus that causes the disease spreads from the soil to the plant in the first twenty centimeters, mainly due to the rain. It penetrates the plant tissue due to wounds in cuts of tillage tolos and galleries made by insects. Micro-organism is present in most high-altitude coffee plantations, with poor drainage and old plantations.

The disease is favored by rain, high humidity, cold temperatures at night (18°C) and warm during the day (28°C). The dispersa lis by the conidia, mycelium, and ascospores which can survive for several years in the soil and in remains of wood colonized by the pathogen. The fungus lesions develop at the base of the stem, in the first twenty centimeters, as a result of the entrance of the fungus by the wounds caused by the weeding and pruning machetes. The attack on the branches is also caused by the entrance of the fungus, product of the spread in the pruning tools, not disinfected for this purpose.

It is common in coffee plantations with good technology to have incidence rates of 10%, which in a population of 5000 plants per hectare translates into a considerable number of plants, which is detrimental to yield per area. If the epidemic has not been dealt with since the beginning, insect populations and sexual structures in the long term, reproduce quickly and the incidence of disease is magnified.

## **Disease management**

The most important strategy that can be applied to manage canker stain is to avoid any form of injury to the tree trunk. The major long-term strategy to control canker stain of coffee is to plant resistant varieties. *Coffea canephora* Pierre ex A. Froehner is reported as resistant, and in *Coffea arabica* L. just one selection, *C. arabica* L. var. Bourbon, has a known resistance to *C. fimbriata sensu lato*. In recent yeares lineages resistant to Ceratocystis canker stain have been bred from either *C. arabica* L. var. Bourbon or *Coffea canephora*, crossed with *C. arabica* var. Caturra (Castro y Cortina, 2002).

The fungus invades the host by disseminating agents, which are the tillage tools, or galleries produced by ambrosial insects when penetrating the host (Brenes, 2018). The best way to avoid the establishment of this fungus is by prevention. It is recommended to avoid causing unnecessary wounds to the tree and to apply fungicide in a preventive way in any cut caused to the trunk or branches, whether in pruning and weeding practices or due to some factor that cracks, tears or causes injury to the coffee tree. It is also recommended to disinfect pruning and weeding tools, several times during the work day. The working tools can be disinfected with various products, some as old as Formol, diluted in 2% water or Sodium Hypochlorite using commercial doses, although currently a wide variety of chemical products are offered to perform an efficient cultural practice. The preventive control of this pathogen is carried out with fungicides from the group of benzimidazoles, chlorothalonil and various triazoles. But there is no fungicides that eliminates the disease once inside the plant tissues. Fungicides should not be aplied in the soil (Castro y Montoya, 1994). Pastes or species of paints based on cupric fungicides can also be used as disinfectants and protectors of pruned plants. All these products should be used as part of an integrated management of the fungus and at the dose recommended by the manufacturer. Disead plants should be eliminated from the field. After the erradication of diseased plants a new seedling can be planted imediately.

Preventive biological control of the pathogen is a practical reality through various species of the fungus Trichoderma spp., which must be used in the field from the production of seedlings and also in the establishment of the new plantation. Ideally, this pathogen should be achieved in the rhizosphere of the coffee crop indefinitely, so that it acts as a true antagonist in this ecological environment.

Other cultural practices that are of great benefit to reduce the intensity of the disease are pruning above forty centimeters and preventing particles of organic matter with the fungus from establishing themselves in the wood. If the plants are infected at the top of the plants it is recomended to cut the the stem 15 cm below the lesión. During the practice of pruning the branches nearby the soil line, it is suggested to cut them, leaving about 2 cm long without causing direct damage to the main stem (Castro, 1998)

It is advisable to remove all the pruned material from the plantation and incinerate it to reduce a significant source of inoculum of the fungus. Plants with the characteristic symptoms of the disease, such as irreversible yellowing, must be eradicated from the plantation, since they constitute a source of permanent inoculum; the hole left by the diseased plant must be solarized for a period of four to eight weeks by placing transparent plastic, to reduce the intensity of propagules in the soil, then a fungicide can be added and finally a program of applying biocontroller fungi is established, to establish balance on the ground (Brenes, 2018).

## Coffee Wilt Disease (Tracheomycosis) Introduction

Coffee Wilt Disease (CWV) or Tracheomycosis is one of the most important coffee diseases, mainly on Coffea canephora var. robusta) in Africa. But the disease was first reported on Coffea liberica W. Bll. ex Hiern var. dewevrei (De Wild. & T. Durand) in 1927 in the Central African Republic (Figuieres, 1941; Felix, 1954; Saccas, 1956). In the mid 1900s the epidemy of CWD was detected in the Central African Republic, Côte d'Ivore, and Democratic Republic of Congo which caused great damage to Coffea liberica and C. canephora Pierre ex A. Froehner. The disease advanced to west and central Africa (Guillemat, 1946); from the 50s onwards it extended to the Ivory Coast, Congo, Ethiopia, Uganda, Nigeria, Sudan, Zimbabwe, Tanzania and Rwanda attacking species of C. canephora and C. arabica (Stewart, 1957; Pieters & Van der Graaff, 1980). In 1970s the epidemics attacked new plantations on C. canephora in the Democratic Republic of Congo and also Uganda and Tanzania. For 25 to 30 years the disease was very severe; soon after the incidence declined with the adoption of sanitary practices and improvement programs. From 1984 onwards again increased the reports of epidemics and economic damage in African countries (Flood, 1996; Girma, 1997; Girma & Hindorf, 2001; Flood et al., 2001). In 1957 CWD was found in C. arabica L. in Ethiopia. The authors suggested that there are variants of the fungus that attacks C. arabica and others C.

*canephora*. The disease can kill the coffee plants 1 to 2 months after the first symptoms. Outside Africa there are no report of CWD. The major impact on coffee production was in Democratic Republic of Congo and Uganda. In Uganda 90% of *C. canephora* farms were affected. In Uganda, annual losses have been estimated at US \$ 3 million. In Ethiopia, losses varied from 44% in Gera to 61% in Bebeka; however, some producers lost about 100% (Girma, 1997). A very good review of the disease in Africa was published by Flood (2021).

## Symptoms

The symptoms of the disease are similar to those caused by *Rosellinia bunodes*, *R. pepo* and *Ceratocystis fimbriata*. The difference is that CWD eliminates the plant with losses in production and increased cost for renovation. Coffee Wilt Disease can manifest at any stage of plant development. Leaves of young plants develop brown necrotic lesions, often along the veins and margins initially. Eventually leaves can dry, shrivel, and fall 3–4 days after the first symptoms. Lower leaves tend to be the first to display sumptoms. In adult plants it is very

common chlorosis, wilting, curling, and drying of the leaves. Unilateral dieback and defoliation can also occurs in the field. Dark brown discoloration of the leaf veins may be apparent. Berries of the diseased plants become red and ripen prematurely. Younger branches develop a dark brown-black necrosis that may also be laterelly restricted. Sometimes the symptoms extend to the entire tree. The key sympom the CWD upon removal the bark is the blue-black staining of the vascular tissues. Discoloration is usually more pronounced toward the stem base. But it may extend from below the soil level to the apex of the trees and may be spiral along stems and branches. Along the trunk bark may become swollen with spirall cracks. Small black perithecia ascomata form under rainy conditions. The perithecia may be visible on the surface of the bark and often in the cracks. Perithecia can be found also on the dead and decaying parts of affected plants. The plants can dies any time between 3 and 15 months after the first external symptoms. But Govindarajan & Subramanian, 1968 pointed out that the plant dies 1 to 2 months after the first symptoms appear. Older and poorly managed trees appear to be more susceptible to the disease (Figure 1, 2 and 3).



Fig 1: Symptoms of the fungus *Gibberela xylariodes* (= *Fusarium xylariodes*) in coffee plants, on the stem and the effect on branches, leaves and grains. (Photos by M. A. Rutherford, Symposium, Fusarium-induces diseases of tropical perennial crop. Current Knowledge of Coffee Wilt Disease, a Mayor Constrain of Coffee Production in Africa).



**Fig 2:** Symptoms of the fungus *Gibberela xylariodes* (= *Fusarium xylariodes*) in coffee plants, on the stem and the effect on branches, leaves and grains. (Photos by M. A. Rutherford, Symposium, Fusarium-induces disease of tropical perennial crop. Current Knowledge of Coffee Wilt Disease, a Mayor Constrain of Coffee Production in Africa).



https://www.google.com/search?q=Fotos+de+Coffee+Wilt+disease+en+Africa&biw=1033&bih=621 &tbm=isch&imgil=N1wQY\_y5jIFWBM%253A

Fig 3: Coffee grower in Africa looking with great sadness and distress at their coffee plants affected by the disease.

## Etiology

Coffee Wilt Disease is caused by Gibberella xylarioides R. Hein & Saccas (anamorph) Fusarium xylarioides (Steyaert: syn F. oxysporum Schltdl.: Fr. Forma xylarioides (steyaert Delassus). Fungal colonies on potato sucrose agar (pH 6.5), are initially pale beige with sparse white mycelium. Purple discoloration may develop with age and be accompanied by dark bluish black, discrete stromata (stomatal initials). Microconidia and macroconidia are produced in slimy masses or short, conidiotenous cells or vegetative mycelium. Microconidia are unicelular, allantoid, curved, and 2.5-3 x 5-10 micrometros. Macroconidia, which tend to be less abundant, are fusoid and falcate, with two to three septa, and measure 4-5 x 20-25 micrometers. Chlamydospores, occasionally produced are oval to globose, smooth or rough, and 8-10 x 10-15 micrometers. Perithecia are globose measure 180-300 x 200-400 micrometers, have a flattaned base, are violaceus in color, and are embedded, singly ou in groups, in dark purple stromata. In the absence of chlamydospores, perithecia may provide a way of survival under adverse conditions. Asci are cylindrical and thin walled, have short pedicels, and measure 7-9,5 x 90-110 micrometers. Asci contain eight monostichous, hyaline to straw-colored ascospores and are fusoid, having one to three spta, are finely roughened, and measure 4,5-6 x 12-14,5 micrometers.

## Epidemiology

Conidia of *F. xylarioides* are transported over long distances by the wind, due to the splash of rainwater and human or animal activities. Transmission from contaminated wood to adjacent uninfected seedlings was confirmed in screen house trials (Phiri and Baker, 2009). Coffee Wilt Disease transmission from contaminated soil to healthy seedlings was also confirmed. Infectivity in soil lasted at least 3 months but then declined. A fallow of 1 year was advised before replanting to avoid reinfection. It seems that insects coffee berry borer, bees or térmites do not transmit the pathogen (Rutherford and Flood, 2005). The pathogen was isolated from banana roots, and the isolates induced symptoms on coffee seedlings (Yet Serani *et al.*, 2007)

The fungus penetrates the trunk due to injuries caused during the execution of cultural practices and by insects. The mycelium

develops inter and intracellularly and progressively invades the xylem. The plant reacts to infection by obstructing the water conduction system through the production of tyloses. The incubation period of the fungus in plant tissues is influenced by age and environmental conditions. The first symptoms vary from a few days to four to six months. The first symptoms indicate general obstruction of the vascular leaves caused by both the fungus structures and the plant's defense reaction. The plant dies after two weeks (Heim & Saccas, 1950).

The life cycle is not well known. The fungus is believed to be a natural inhabitant of the soil; however, the fungus does not live long time in the soil as it rarely produces resting structures called chlamydospores (Van der Graaff & Pieters, 1978). Gibberella xylarioides R. Hein & Saccas is considered a soilborne fungus that infects, colonizes, and induces symptoms in a manner similar to that of other vascular wilt pathogens. Ascospores produced on the basis of diseased plants and also on dead cultural branches and stem could act as survival structures in the soil. The fungus can also live on plant residues or on alternative hosts. In Uganda the fungus was isolated from banana roots however it needs to be proved. The primary mode of penetration into the vascular tissues is by the roots directly. How and the time of survival of G. xylarioides in the soil is also unclear. The fungus penetrates the roots also through wound sites and lower stem that form naturally or result from farming practices. Then the fungus colonizes the vascular tissue of the xylem with the blockage of the vessels and toxin production, leading to the wiltlike symptoms and discoloration observed in wood. Microconidia, macroconidia and ascospores are presumably dispersed by air, water, and human activity. Soil and plant debris, particularly wood, are importante sources of inoculum and disease spread. Wood cuttings used for vegetative propagation are exchanged and sold by growers and nurseries to establish new plantings. The efforts to isolate the fungus from infected coffee berries has been unsuccessful. G. xylarioides is restricted to Coffea spp.

There is no information in the literature on the effect of the environment on the disease. The germination of the conidia is favored at a temperature of 30 <sup>o</sup>C and does not require liquid water on the leaf surface, only a very humid atmosphere. Under laboratory conditions the maximum growth of the fungus was at

25 <sup>o</sup>C in culture medium. Stress produced by the shade of tree plants and marginal areas of low fertility are conditions that favor the disease (Siddiqui, 1965). The disease is more severe in old and under-fertilized plants.

It is very likely that conidia and ascospores are spread by wind, rain, human activities, pruning and harvesting (Jacques, 1954). Wind is the importante way for dissemination of the conidia and ascospores at a long distance. Cultivation practices and splashing raindrops redistribute the pathogen in the coffee plantations (Siddiqui & Corbett, 1968).

Spores can germinate in moist environments without the presence of liquid water. Penetration occurs through injuries to the plant cortex. Wounds during cultural practices can serve as a way for penetration of the fungus in the plant tissues (Krantz & Mogk, 1973). The transmittability of the fungus by the seeds still needs confirmation.

## **Disease management**

The most important measure of disease control is to employ resistant varieties. In the 50s and 60s when the disease was very severe in Central and East Africa, *C. canephora* was the basis for breeding programs aimed at resistance to the disease. In Ethiopia, it was found differences between *C. arabica* L. lines to the disease (Van der Graaff & Pieters, 1978). Such authors suggested that resistance is quantitative in nature in *C. arabica*. There is no evidence of qualitative resistance. But the mechanisms of resistance in coffee plants remains unclear.

In 2001 researchers from Jima Station in Ethiopia demonstrated that coffee varieties resistant to CBD are at some level resistant to CWD (Girma & Hindorf, 2001).

In the absence of resistant varieties the efficient measure to prevent the disease is to adopt the exclusion of the pathogen into disease-free areas. This may be achieved by strict quarentine measures to control the distribution of seeds, vegetative cuttings, clones, seedlings and plantlets produced by nurseries, and soil from the affected areas. New plantings should be established with disease-free material. Others measures to minimize the severity of the disease in the field are: maintaining a vigorous and well-managed crop, minimize the physical damage to coffee trees to reduce the risk of infection and delay subsequente disease development. Avoid wound the stem with cultural measures is very importante to prevent the disease. Cultural measures are also very important when the disease is already installed in the planting areas. Cultural practices included frequent inspection of the crop, along with uprooting and burning of infected material (in situ). Removal of coffee bushes ahead of the infection (to reduce spread between plantations) was considered effective in Cote d'Ivoire; gaps of a few hundred metres were considered

enough to confine the disease (Deassus, 1954). Measures such as reducing the source of inoculum by burning the diseased plant on the spot and also the adjacent plants even if you have no symptoms of the disease. In order to prevent the spread of the fungus in the planting fields, it is recommended to leave a 300 m strip around the site from where the sick plants were burned free of plants. Preventing injury to plants and treating pruning tools with disinfectant products before use and between the pruning process.

In infected areas, further spread and crop loss may be reduced or prevented by adopting phytosanitary measures, including in situ removal and destruction, by burning or burying of affected trees that provide foci of infection. Coffee may be replanted with resistant coffee (if available) or an alternate crop or left fallow for at least one year. The planting of non-host plants is recommended to reduce the population of the fungus in the soil. Coffee plant materials and soil should not be moved within or from affected areas. Coffee wood should not be stored as a source of firewood. Farm implements particularly those used for pruning and weeding around coffee trees, should be routinely cleaned with a suitable disinfectants or fungicide. Care should be taken not to damage the trees because wounds may provide entry points for the fungus. Vigilance is required by growers and local national authorities to monitor disease progress in the field, identify and tackle new outbreaks at an early stage of development, and enforce required management measures.

## **Concluding remarks**

The two soil borne disease most important of coffee are Ceratocystis canker stem (Ceratocystis fimbriata) and Coffee Wilt Disease (Fusarium xylarioides). The first occur in Central and South America, except Brazil and the second is prevalent only in the Africa continent. The main method of control of these diseases is by resistant varieties. For the other two diseases Rhizoctoniosis (Rhizoctonia solani) and Rosellinia root rot (Rosellinia bunodes) there are not any source of coffee resistant germoplam to be bred and to select. Rosellinia root rot is not a problem in Brazil due to the fact that all coffee plantations is growing under sun light and it is forbiden to devaste the forest to growth coffee. In Central America this disease is responsable to kill coffee plants because all coffee plantations in growing under shade tress susceptible to the disease. The coffee breeders of the American continent should be aware of the great importance of Ceratocystis canker stem and Coffee Wilt Disease. There are source of coffee germoplasm resistant to both diseases in Africa continent. Also the government authorities should be aware of the great importance of this disease. Seeds of coffee must be prohibited to enter in the America's country because they can disseminated these diseases.

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