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BIOLOGY OF *COLLETOTRICHUM* SPP. AND EPIDEMIOLOGY OF THE ANTHRACNOSE IN TROPICAL FRUIT TREES[1]

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ABSTRACT- The anthracnose is important disease in the pre and postharvest phases. Several species of *Colletotrichum* (*C. gloeosporioides, C. acutatum, C. musae e C. magna*) are responsible for inciting this disease. The pathogen infects many fruit trees in tropical and temperate regions, causing considerable damage and loss in all phases of cultures. Characteristic symptoms are dark necrotic lesions depressed, subcircular or angular shaped, and there may be coalescing. Infections have a special feature: the phenomenon of quiescence. This process has important implications, particularly in post-harvest, because the damage from infections reflect only this phase. The intensity of the disease have been striking at temperatures from 24 to 28 °C and in the presence of high relative humidity. The understanding of some aspects of the biology of the pathogen (the process of quiescence) and the epidemiology of the disease is crucial, since much has not yet been fully clarified, especially when the aim is to achieve sustainable management.

Keywords: Quiescence. Preharvest. Postharvest. Fungal disease.

BIOLOGIA DE *COLLETOTRICHUM* SPP. E EPIDEMIOLOGIA DA ANTRACNOSE EM FRUTEIRAS TROPICAIS

RESUMO - A antracnose é uma doença importante nas fases de pré e pós-colheita. Diversas espécies de *Colletotrichum* (*C. gloeosporioides, C. acutatum, C. musae e C. magna*) são responsáveis por incitar esta enfermidade. O patógeno infecta várias fruteiras em regiões tropicais e temperadas, ocasionando danos e perdas consideráveis em todas as fases das culturas. Os sintomas característicos são lesões necróticas escuras deprimidas, com formatos subcirculares ou angulares, podendo haver coalescimento. As infecções apresentam uma particularidade: o fenômeno da quiescência. Este processo tem implicações importantes, principalmente na pós-colheita, pois os danos refletirão apenas nesta fase. A intensidade da doença tem sido marcante em temperaturas de 24 a 28 °C e, na presença de alta umidade relativa. O entendimento dos aspectos relacionados com a biologia do patógeno (processo de quiescência) e a epidemiologia da doença é fundamental, pois muito ainda não foi totalmente esclarecido, principalmente quando o intuito é realizar um manejo sustentável.

Palavras chave: Quiescência. Pré-colheita. Pós-colheita. Doença fúngica.

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INTRODUCTION

Colletotrichum spp. is an important pathogen in the tropics and the sub-tropics. It is responsible for causing one of the most significant diseases in the agricultural crop systems, anthracnose. Symptoms of anthracnose occur in fruits, branches and leaves of several crops of agronomic interest. Anthracnose symptoms include dark depressed lesions, that are semi-circular or angular shaped, where coalesce can occur. In advanced stages, an eruption of an orange to pinkish colored conidia mass occurs (JEFFRIES et al., 1990). There is an intense production of *Colletotrichum* conidia on branches, leaves, flowers and fruit, becoming the primary inoculum of the disease. These spores can be released and dispersed through splashes of water to other parts of the plant resulting in secondary inoculum. The main source of inoculum in fruit is most likely infected leaves. In conditions of high humidity, conidia is released to immature fruit (FITZELL; PEAK, 1984). Infections caused by the *Colletotrichum* species become important in the post-harvest season as this pathogen presents quiescence. The quiescent infections occur during pre-harvest, in the flowering and fruit development stages; however, the reflection of the infection is highlighted in post-harvest. The quiescent infections are a response to adverse conditions of the host pathogen. Conditions such as lack of nutrients and important enzymes for the pathogens, as well as the presence of preformed antifungal compounds are related to quiescence. The presence of the hormone ethylene in the ripening process of the fruit is essential (PRUSKY, 1996). Understanding the dynamics of this process is important because the economic cost of quiescent infections is very high, resulting in the involvement of the entire production chain from harvest, transport, storage to packing.

Studies involving epidemiological variables allow a better understanding of *Colletotrichum* pathosystems/tropical fruit, generating information for more sustainable management of anthracnose (FREEMAN et al., 1998). Therefore, this review will report some features of the biology of the pathogen and especially the epidemiological aspects of the disease in various fruit species of agronomic interest.

THE MAIN COLLETOTRICHUM SPECIES AND ITS EPIDEMIOLOGICAL IMPLICATIONS

Some species of *Colletotrichum* are responsible for causing anthracnose in tropical fruits. Among the species that cause the most harm, the following stand out: *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc, (*Glomerella cingulata* (Stonem.) Spauld. & Von Schrenk), *Colletotrichum acutatum* Simmonds and *Colletotrichum musae* (Berk. & Curtis) Arx. (BAILEY; JEGER, 1992).

The fungus *C. gloeosporioides* is responsible for inciting various diseases in different hosts. The most typical disease is anthracnose in fruits, leaves and branches. Other diseases are associated with this causal agent, such as dieback, root rot, flowers and fruits, leaf spots and burning seedlings. Studies using molecular tools showed that this species is not always responsible for anthracnose. There are two complexes: the "*gloeosporioides*", which includes species with cylindrical conidia and the "*acutatum*" with fusiform conidia (PHOULIVONG et al, 2010). However, *C. acutatum* conidia shows variation in shape (not always a spindle) which has caused confusion in the classification, and is often referred to as *C. gloeosporioides* (DAMM et al., 2012a). The use of molecular tools (phylogenetic analysis), together with morphological markers (conidia and appressoria morphology, development of perithecia with asci and ascospores), cultural (coloring of the colonies) and physiological (mycelial growth) is an alternative to minimize possible mistakes in the classification of the *Colletotrichum* species (DAMM et al., 2012a; WEIR et al., 2012). In addition to *C. gloeosporioides*, there is evidence that *C. acutatum* (PERES et al., 2005; DAMM et al., 2012a.) and *C. magna* (NASCIMENTO et al., 2010) cause anthracnose. Another cited species is *C. musae*, where there is the phenomenon of host specialization in bananas (PLOETZ et al., 2003).

The identification of the species responsible for anthracnose and the understanding of cross-infection occurring in fruits is of paramount importance for epidemiological studies and disease management (FREEMAN et al., 1998). In tropical conditions, many of the fruit trees are grown in close proximity and the potential for dispersal of propagules from one host to another is huge (FREEMAN et al., 1998). LAKSHMI et al (2011) found that isolates from mango trees presented infection in other fruit species such as cashew and guava. Another interesting fact is that a species can be host to many species of *Colletotrichum*, which causes some confusion when using the host as taxonomic criteria (PHOULIVONG et al., 2010). Therefore, understanding the behavior of cross-infection in tropical fruits is essential for the management of anthracnose in pre and post-harvest strategy.

BIOLOGY AND EPIDEMIOLOGY OF ANTHRACNOSE

Anthracnose is an important disease in fruit trees, causing serious damage, especially in the post-harvest stage (ARAUZ, 2000). The correct determination of the etiology of anthracnose caused by *Colletotrichum* species is essential for understanding the epidemiology of the disease (FREEMAN et al., 1998). The epidemiological processes of the anthracnose and the pathogen biology have some particularities that are inherent to each pathosystem. The following are the main epidemiological processes and biology of pathogens in different fruit species.

AVOCADO TREE (*Persea americana* Mill.) - Anthracnose causes serious losses during the post-harvest stage. Until recently, *Colletotrichum* species were responsible for *anthracnose C. gloeosporioides* and *C. acutatum* (MENGE; PLOETZ, 2003). However, phylogenetic studies using molecular markers, showed another species responsible for this disease (SILVA-ROJAS; AVILA-QUEZADA, 2011). In other studies, it was also found that *C. fioriniae* and *C. aenigma* are responsible for causing anthracnose in avocado trees. Isolates from Australia and Israel were used with the aid of molecular (phylogenetic analysis of ITS, GAPDH, CHS-1, HIS3, ACT and TUB2 for *C. fioriniae* and ITS, GAPDH, CAL, CHS -1, GS, SOD2, ACT and TUB2 for *C. aenigma*), morphological and cultural tools proved that this species was responsible for causing the disease (DAMM et al., 2012a). As in other pathosystems, the occurrence of quiescent infections is remarkable in avocado trees. The quiescence is caused by the presence of a preformed antifungal compound: diene (PRUSKY, 1996). The spread of the disease occurs primarily by splashes of water and wind (FITZELL; PEAK, 1984). Spores can infect all parts of the plant, but flowers and fruits are often more susceptible to the pathogen (MENGE; PLOETZ, 2003). The intensity of the disease is favored under conditions of high humidity and temperatures between 24 and 28° C, and symptoms occur in all parts of the plant (Table 1). The favorable conditions for the infectious process are the presence of injury, rain, hail, strong winds and extreme heat. The pathogen survives in a saprophyte manner on dead branches, old injuries, fruits and remaining parts in the soil, which sporulates when there are conditions of high temperature and humidity.

ACEROLA TREE (*Malpighia emarginata* Sessé & Moc ex DC.) - Anthracnose causes considerable damage at all stages of development of the acerola tree. The *Colletotrichum* species responsible for the disease are *C. gloeosporioides* and *C. dematium* (Pers. Ex Fr.) Grove (ALMEIDA et al., 2003). *Colletotrichum spp*. can infect the plants throughout the year, and the symptoms are very characteristic (Table 1). The pathogen evolves in the range of temperature between 24 and 32°C. The intensity of the disease occurs in periods of higher rainfall, indicating the importance of rain in the dispersal of the pathogen. In nurseries, the infection is more harmful because of favorable conditions, such as availability of young tissues, proximity to plants and higher humidity levels. On the field, anthracnose persists for a longer period in irrigated plantations, and is commonly found on canopy leaves (FREIRE; CARDOSO, 2003).

BANANA TREES (*Musa X paradisiaca* L.) - Infections occur in both the pre and post-harvest in banana plantations. The *Colletotrichum* species responsible for anthracnose is *C. musae* (PLOETZ et al., 2003). The infectious process occurs during flowering and fruit development. Symptoms may come from lesions of the infections occurring in green fruit, which remain quiescent until the fruit ripens (CHAHAKRAVARTY, 1957) (Table 1). Spores produced in old leaves, as well as crop residues are released by water and dispersed by wind or insects (FITZELL; PEAK, 1984). Ecological variables such as temperature and humidity are extremely important to the epidemics of anthracnose. In the presence of free flowing water, conidia germinate within 4 to 24 hours to form the appressoria. During penetration within 24 and 48 hours, there is a hypersensitive reaction to adjacent epidermal cells (CHAHAKRAVARTY, 1957). There are a few methods for early detection of quiescent infections of anthracnose in bananas. For example, the use of ethylene to “break" the quiescence occurs during the reactivation of the infection of the fruit ripening process (LAPEYRE DE BELLAIRE et al., 2008). Detection of quiescent infections has great importance for epidemiological studies because the treatment is carried out during pre-harvest and can be assessed and performed efficiently. Furthermore, the understanding of the influence of ecological variables results in useful information for development of forecasting systems and, consequently, on decision making for more sustainable measures to control anthracnose of the banana tree.

CASHEW TREE (*Anacardium occidentale* L.) - The *Colletotrichum* species responsible for anthracnose is *C. gloeosporioides* (FREIRE et al., 2002). The symptoms are characteristic (Table 1) and the pathogen survives in infected tissues internally and in crop remains. The dispersal of propagules occurs through splashes of water or wind. The presence of free flowing water in the infectious process is important because it allows the decoupling of conidia as well as eliminates the self-inhibitory effect of mucilage. Spores emit a germ tube, and one appressorium is subsequently formed in response to a physical and chemical stimuli host (JEFFRIES et al., 1990). The penetration occurs directly, breaking the cuticle of the host cell with the aid of enzymes (BAILEY; JEGER et al., 1992). In the northeast, during the rainy season, anthracnose severity reaches high levels. Temperatures between 21-29 °C and at least 10 hours of wetness conditions are favorable for the disease (FREIRE et al., 2002). The occurrence of cross-infection in orchards is a reality. Management

Table 1. Symptoms of Anthracnose in Tropical Fruit.

Host	Main Symptoms/Parts of the Plant
Avocado Tree (MENGE; PLOETZ, 2003)	Leaves: Brown stains in the margins or between the veins. Branches: Injuries similar to that of leaves may occur near new branches. Flowers: Dark injuries causing death and abortion of fruits. Fruits: Dark, depressed and isolated injuries. Soft rot.
Acerola Tree (FREIRE, CARDOSO, 2003)	Leaves: Circular spots, white like color, with a depressed center. Rarely coalescing. Disruption of necrotic tissue. Brown halo surrounding the injured areas. Branches: Drought descendant of young branches (“dieback "). Flowers: Necrotic lesions. Fruits: Dark circular lesions, depressed and coalescing.
Banana Tree (PLOETZ et al., 2003)	Leaves, Branches and Flowers: Necrotic lesions. Fruits: immature fruits- dark spots with white like colored halo. Ripe fruits- “coffee” color stains, depressed and coalescing.
Cashew Tree (FREIRE; CARDOSO, 2002)	Leaves: Necrotic, irregular brown lesions. Red lesions on older leaves, with occurring perforations. May become twisted, blackened and fall. Branches: Necrotic lesions. Flowers: Injuries in inflorescence, abortion and drought. Fruits: Dark depressed lesions. Cracks, decay and premature fall.
Citrus Trees (LARANJEIRA et al., 2002)	Leaves: Necrotic lesions. Branches: Dieback. Necrotic lesions. Flowers: Aqueous, dried and coalescing lesions. Abortion of flowers. Fruits: Young fruit-chlorotic and fall. Basal disc, cup and retained peduncle. Mature fruits- dry and rounded depressed lesions, reaching the shell.
Guava Tree (LIM; MANICOM, 2003)	Leaves: Dark circular shaped lesions. Branches: Blight of new branches. Red branches, becoming dark brown, dry and brittle. Flowers: Necrotic lesions. Fruits: Immature fruit- circular dry lesions, pustules in a canker form, may coalesce. May become mummified and black. Mature fruit- light brown, depressed and soaked lesions.
Soursop Tree (CARDOSO; FREIRE, 2003)	Leaves: Lesions with a coalescent gray/light brown center. Branches: Death and necrotic lesions on shoots or branches pointers. Death of the grafts. Flowers: Death of pointers branches (dieback). Fruits: Immature fruits-dark, mummified. Mature fruits-circular, dark, and deep coalescent lesions. There may be cracks.
Papaya (DICKMAN; ALVAREZ, 1983)	Leaves: Petiole-dark lesions. Leaves: Lesions are less frequent. When they occur, they are circular, have irregular borders and have a grayish center. Branches and Flowers: Necrotic lesions. Fruits: Rounded and deep lesions. Lesions may coalesce, deepen and detach, leaving holes in the fruit.
Manga Tree (ARAUZ, 2000)	Leaves: Brown, rounded or irregular lesions. With the increase in lesion size, coalescence and breakup of the limb occurs. Branches: Dark and necrotic lesions. Branches dry and darken from the tip to the base. Flowers: Deep dark spots, causing the death of flowers. Fruits may fall. Fruits: Dark, depressed, variable sized and generally round lesions. Lesions may coalesce to involve the whole fruit, sometimes causing cracks in the bark.

strategies for this disease should also consider other potential hosts of the pathogen fruit trees.

CITRUS TREES (*Citrus sinensis* (L.) Osbeck) – The premature fall of the citrus is caused by the fungus *C. acutatum* and causes severe epidemics only in years when there is rain during bloom (LARANJEIRA et al., 2002). While the rot in the fruit, which occurs, caused by *C. gloeosporioides* is an important disease in postharvest (FREEMAN et al., 1998). The symptoms of rot occurs in fruits, leaves and flowers (Table 1) (PERES et al., 2005). The pathogen has an infected quiescent and subsequently no production of conidia that are dispersed by splashing will infect the petals and fruits (Figure 1).

The favorable conditions for infection are 10 to 12 hours of leaf wetness and temperatures between 24-28°C (LARANJEIRA et al., 2002). PERES et al. (2002b) developed a PFD - FAD system for decision making for the premature fall of citrus. In this system, the history of disease in the orchard, the susceptibility of the host, the blossoming stage, the presence of rain, the wetness period and inoculum levels is evaluated. The risks of the epidemic are estimated and determine the correct period of application of fungicides to control the disease.

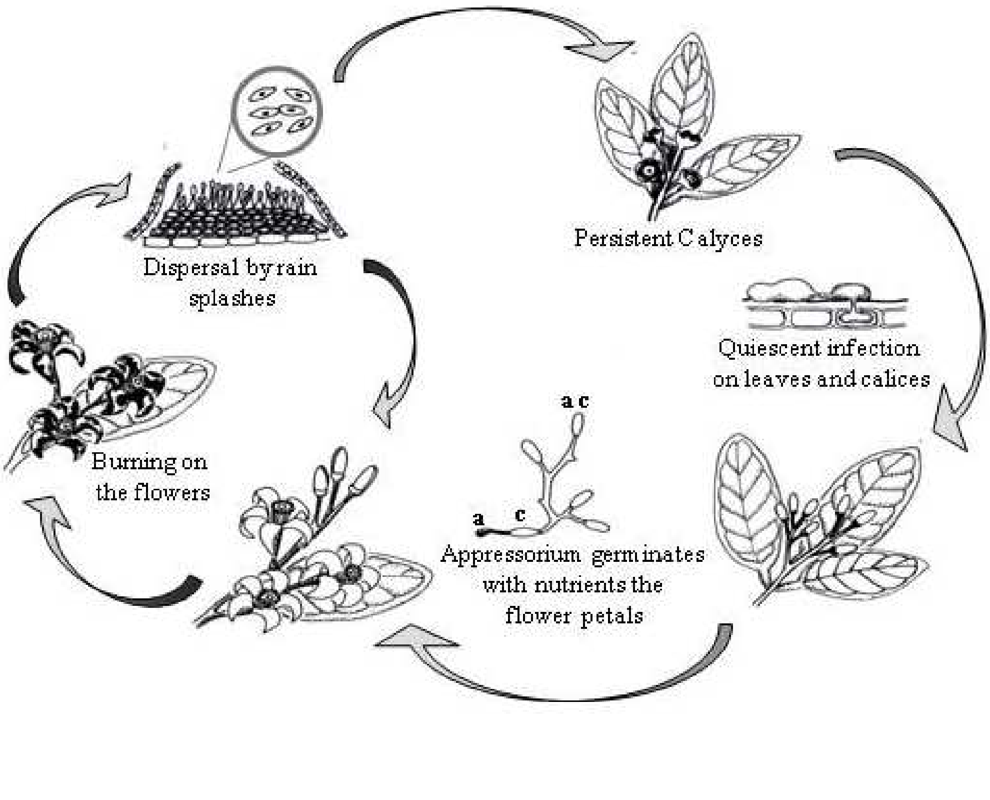

Figure 1. Life cycle of *C. acutatum* on citrus (adapted from PERES et al., 2005)

GUAVA TREES (*Psidium guajava* L.) - The *Colletotrichum* species responsible for anthracnose is *C. gloeosporioides* (LIM; MANICOM, 2003). In tropical conditions, it was found that the species *C. acutatum* is also associated with the lesions of anthracnose (PERES et al., 2002a). In other studies it was found that *C. guajavae* is also responsible for causing anthracnose in guava fruits. Were used isolates from India and with the help of molecular (phylogenetic analysis of ITS, GAPDH, CHS-1, HIS3, ACT and TUB2), morphological and cultural tools proved that this species was responsible for causing the disease (DAMM et al., 2012a). Symptoms of the disease occur in all parts of the plant (Table 1). The penetration of the pathogen occurs through wounds, as well as the flower opening. Frequently in the formation of the appressorium, the pathogen presents a quiescent infection, until the present conditions are favorable for the restoration of infection (PRUSKY, 1996; LIM; MANICOM, 2003). Temperatures of about 30°C, as well as long periods of wetness favor the infection process (LIM; MANICOM, 2003). Characterizing the level of inoculum in guava orchards and monitoring environmental conditions, as well as understanding the process of quiescence is essential for disease management in the field.

SOURSOP TREE (*Annona muricata* L.) - Anthracnose, when it focuses on the flowering and fruiting, causes severe losses in soursop trees. The *Colletotrichum* species responsible for the disease is *C. gloeosporioides* (PLOETZ, 2003). Symptoms occur in all parts of the plant (Table 1). Spores are dispersed by splashes of water and wind. After adhering to the surface of the host, they germinate and begin the infectious process. There is formation of hyphae (*peg*) penetration, but the infection remains quiescent until the conditions become favorable occurring at the beginning of fruit ripening (PRUSKY, 1996). The pathogen survives in dry branches, old injuries, fruit and crop residues. Long periods of rain and overcast skies, as well as the intense nighttime dew conditions are favorable for disease development. This period coincides with the flowering and fruiting time of the crop. Other conditions are also conducive to disease, such as night temperatures between 20 and 24°C, inadequate fertilization and pest attack (CARDOSO; FREIRE, 2003).

PAPAYA TREE (*Carica papaya* L.) - The pathogen commonly responsible for inciting anthracnose is *C. gloeosporioides* (BAILEY, JEGER, 1992). However, recent studies showed that *C. magna* is responsible for complex diseases (anthracnose, chocolate spot and stem end rot) which causes considerable damage and losses in yield (NASCIMENTO et al., 2010). In other studies, it was found that *C. acutatum*, *C. simmondsii* and *C. karstii* are also responsible for causing anthracnose in papaya trees. Were used isolates from Tanzania, Australia and Brazil, with the help of molecular (phylogenetic analysis of ITS, GAPDH, CHS- 1 , HIS3 , CAL, ACT and TUB2 for *C. acutatum* and *C. simmondsii* and ITS , GAPDH , CHS-1 , HIS3 , CAL , ACT and TUB2 for *C. karstii*), morphological and cultural tools it was proven that these species were responsible for causing the disease (DAMM et al., 2012a,b). Symptoms of anthracnose occur in branches, leaves, flowers and fruits, which may cause coalescence of lesions (Table 1) (DICKMAN; ALVAREZ, 1983). Under high humidity and temperatures above 22°C the production of orange mucilage, with hyaline conidia was observed in the center of the lesions (BAILEY, JEGER, 1992). The *Colletotrichum spp*. infection is characterized by the quiescence phenomenon that is hampering epidemiological studies. The conidia clinging to the surface of the host, germinates, produces melanized appressoria and infective hyphae, but the pathogen colonization is reduced. With physiological maturation, it is believed to have antifungal compounds, reducing sugar increase, and other compounds, which are favorable to reactivation of infection (PRUSKY, 1996). The infected fruit and senescent leaves represent important sources of inoculum. The ascospore production in petioles of dried leaves has often been observed, but the importance of the sexual stage of the pathogen is not yet fully understood. The drops of rain and splashes of irrigation water are the main sources of disease dissemination (FITZELL; PEAK, 1984).

MANGA TREE (*Mangifera indica* L.) - is a disease that occurs in all producing regions of the world. The species responsible for inciting disorder is *C. gloeosporioides* (ARAUZ, 2000). In recent studies, it was found that *C. simmondsii* and *C. asianum* are also responsible for causing anthracnose in mango trees. Were used isolates from Australia, the Philippines and Thailand, with the help of molecular (phylogenetic analysis of ITS, GAPDH, CHS- 1, HIS3, ACT and TUB2 for *C. simmondsii* and ITS, GAPDH, CAL, CHS- 1, GS, SOD2, ACT and TUB2 for *C. asianum*), morphological and cultural tools proved that these species were causal agents of disease (DAMM et al., 2012a). The infection begins after flowering or in the last stages of fruit development, during wet periods. The host tissue can invade directly, with the aid of the appressoria or by injury. However, under unfavorable conditions, the pathogen remains quiescent (PRUSKY, 1996). The symptoms can be observed in all parts of the plant (Table 1). Spores require humidity above 95 % and temperatures between 20 and 30°C for germination to occur and for the formation of appressoria (FITZELL; PEAK, 1984). Spores are produced in acervulus on sick leaves, bare terminal branches, mummified inflorescences and bacterial lesions that surround the developing inflorescences. If the relative humidity is between 95 and 97% during flowering, the burning of the panicles occurs and therefore no setting of fruits. Propagules are released and dispersed when acervulus are wet. They are transported by rain and wind, or upon contact with insects, other animals and tools. So far, we found no involvement of the ascospores of *G. cingulata* the infection (JEFFRIES et al., 1990). During the rainy season, anthracnose severity is higher in young leaves and inflorescences. Large amounts of spores are sampled during prolonged rainy periods and these periods are positively correlated with flowering and more severe damage of the disease (BAILEY; JEGER, 1992) (Figure 2). Regarding the development of predictive models of disease, FITZELL et al. (1984), developed a model based on climatic variables for estimating infection levels of *C. gloeosporioides* and defining the best control strategies.

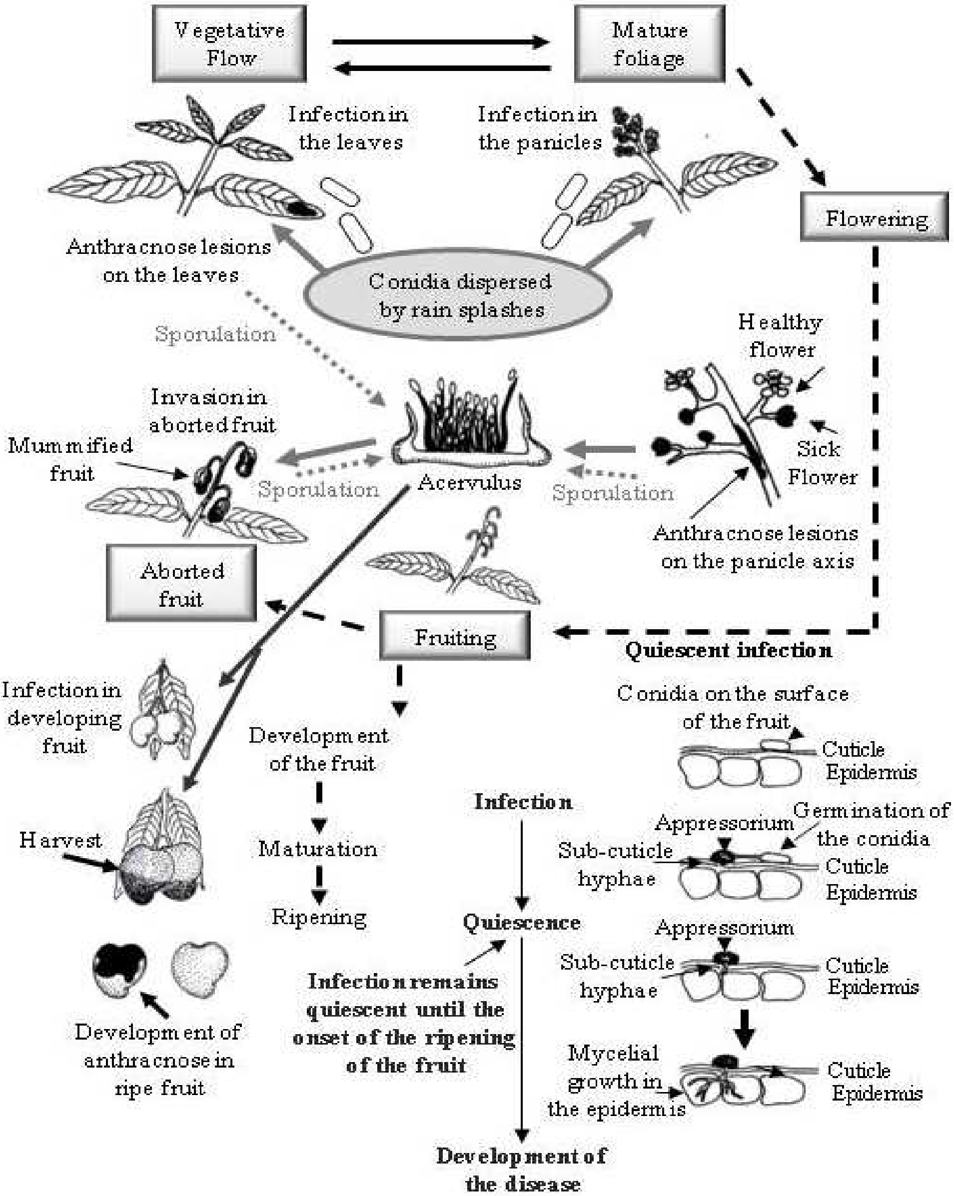

Figure 2. Cycle of anthracnose of the mango tree (adapted from ARAUZ, 2000)

CONCLUSION

Several species of *Colletotrichum* are responsible for anthracnose in tropical fruit trees. Many of these fruit trees are grown in close proximity within the orchards, facilitating the dispersal of propagules between plants and the risk of epidemics is huge. This study made the understanding of the biology of the pathogen and the epidemiology of anthracnose in different fruit species of agronomic interest possible. The infection process of *Colletotrichum spp*. occurs in immature fruit, resulting in the phenomenon of quiescence. The tax cost for these infections is high, as reflected in postharvest. Moreover, environmental variables such as temperature and humidity are extremely important for the development of the infectious process. Therefore, the knowledge summarized in this study is fundamental when one's final strategy is sustainable management of anthracnose in various tropical fruit trees.

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