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Presence of phytopathogenic fungi and oomycetes on rice and avocado crops in Tolima (Colombia)

Vinchira-Villarraga Diana Marcela[1], Macias-Camacho Jeimy[1], Mendez-Olivera Juan David[2], Méndez-Tibambre María Elizabeth[1], Rodríguez-García Victoria[2], Saavedra-Orduz Zeidy[2], Torres-López Michael Alejandro[2] and Moreno-Sarmiento Nubia[1]*

[1]Instituto de Biotecnología, Universidad Nacional de Colombia, Sede Bogotá, Edificio Manuel Ancizar 224, 111321, Bogotá, Colombia.
[2]Biocultivos S. A. Departamento Técnico, Cra. 16 Sur No 67 – 406. Parque Industrial. 730005, Ibagué, Colombia.

Tolima is one of the largest zones in Colombia where avocado and rice are produced. The disease management programs for these crops are mainly chemical-dependent and tend to have variable results since the diagnosis of some diseases is based on unspecific symptoms. In this context, the present study aims to bring information about the phytopathogens affecting Tolima´s rice and avocado crops. From foliar tissue, culm, and roots of rice plants, 18 isolates closely related to *Gaeumannomyces graminis var. graminis/G. oryzae* and 06 *Magnaporthe oryzae* were obtained. The isolation of these fungi was concordant with the crown sheath rot and rice blast symptoms observed. Also, 13 different fungi belonging to the *C. acutatum* and *C. gloeosporioides* complex were isolated from avocado fruits with symptoms of anthracnose. Interestingly, only two *Phytophthora* sp. isolates were purified from necrotic roots of avocado trees. However, several fungi belonging to the *Cylindrocarpon/Ilyonectria* genera and one strain identified as *Bjerkandera adusta* were isolated from these samples. Symptoms caused by these fungi are similar making it necessary to do a differential diagnosis of the actual pathogen causing avocado root necrosis. The data obtained constitute the first report of the above-mentioned phytopathogens in Tolima avocado crops and established the basis for an epidemiologic study of its distribution in Colombia.

Key words: Rice, avocado, *Gaeumannomyces, Magnaporthe, Phytophthora, Cylindrocarpon, Ilyonectria, Colletotrichum.*

INTRODUCTION

Rice and avocado are two of the most relevant crops in Colombia either for internal consumption (rice) or as an exportation product (avocado). In 2019, 1.904.819 tons of rice and 210.280 tons of avocado were produced (Dane-

*Corresponding author. E-mail: ncmorenos@unal.edu.co.

Fedearroz, 2020; DANE, 2020). Tolima has the first and second-largest area cultivated with these crops in Colombia (51.189 ha. for rice and 21.115 ha for avocado). In the first six months of 2019, Tolima contributed 18.19 and 21.6% of the production of avocado and rice, respectively. However, in the case of avocado crops, its yield (2.27 tons/ha) was lower than other departments with smaller production areas such as Antioquia (3.98 tons/ha) and Valle del Cauca (9.6 ton/ha) (DANE, 2020) leading to a less participation in avocado exportation.

On the other hand, lack of crop rotation, exclusive use of moist conditions for rice grown, and environmental conditions such as high humidity and temperature characteristics of Tolima favor the development of several phytopathogenic fungi being one of the reasons related to the rice yield reduction in Tolima (Echeverri-Rico, 2016). In rice crops, more than 70 different pathogens have been reported including several soil-borne fungi (Ke et al., 2016). Two of these fungi are wide distributed and have a significant impact on rice production: *Gaeumannomyces graminis* var *graminis*, the causal agent of crown sheath rot (or sheath blight) and *Magnaporthe oryzae* (teleomorph) (Herbert) Barr (anamorph: *Pyricularia oryzae*) the causal agent of rice blast (Zhang et al., 2016).

Gaeumannomyces graminis (Sacc.) von Arx & D. Olivier var *graminis* (Ggg) is a soil-borne phytopathogen that colonizes the roots and culms of rice (Hawerroth et al., 2017). This pathogen generates typical dark brown and black lesion on rice sheath and culms and necrosis on rice roots which could lead to early grain maturation and reduce the number of grains per panicle (Peixoto et al., 2013). Even though it has been reported that this pathogen causes minor loses in production (Hernández-Restrepo et al., 2016), its incidence in crops is high in several regions including Brazil (Peixoto et al., 2013), Texas, Florida (USA) (Datnoff et al., 1997) and Colombia where 34 to 99% incidence is reported (Echeverri-Rico, 2016).

Magnaporthe oryzae (previously classified as *M. grisea*) is a hemibiotrophic fungus described as the most devasting fungal pathogens in rice crops, causing annual production losses of 10 - 30% (Ebbole, 2007; Zhang et al., 2016). This pathogen was first reported in China (1637) and Japan (1704) (Kumar and Ashraf, 2019) and has caused several outbreaks in rice cropping regions of Italy (Kunova et al., 2014; Titone et al., 2015), Kenya (Kihoro et al., 2013), Bangladesh (Callaway, 2016) and Australia (Fang et al., 2017); it is used as a model for the study of plant-pathogen interaction and development of plant resistance.

Rice blast is characterized by the production of diamond-shaped lesions on leaves and, to a lesser extent on leaf collars, necks, panicles, pedicels, and seeds (TeBeest et al., 2007). The symptoms on leaves usually appear during tillering and could be followed by neck

blast lesions that are correlated with higher yield losses (Ghataka et al.,2013). Because of that, an early diagnosis is necessary to prevent the negative impacts of this fungus on rice production.

Avocado crops are also affected by a large number of microbial phytopathogens. However, the higher reductions in avocado yields are probably caused by the oomycete *Phytophthora cinnamomi var. cinnamomi* Rand (Hardham and Blackman, 2018). Root rot, a disease caused by *P. cinnamomi*, has been reported in more than 56 countries and was described since 1927 (Tucker, 1929). Root rot affected 60-75% of avocado orchards in California (USA) and had presented serious outbreaks that caused economical losses of almost USD$40 million in 1989 (Belisle et al., 2019; Pagliaccia et al., 2013; Ploetz, 2013). In Australia, *P. cinnamomi* caused production losses up to 50% in 1974 and is to date, one of the most limiting factors of avocado production in this country (Ploetz, 2013; Salgadoe et al., 2018). On Antioquia (Colombia) fields, root rot incidence could cause economical losses of 356 USD/nursery and 2340 USD/ha showing the impact that this pathogen can cause on this crop (Ramírez-Gil et al., 2017).

During the fruit development and postharvest time, avocados are also susceptible to the attack of different fungi including those belonging to the *Colletotrichum* genus. This Ascomycota phytopathogen is classified as a hemibiotrophic fungus and is the causal agent of anthracnose disease in a wide range of host plants (Phoulivong, 2011). Several species of *C. gloeosporioides, C. boninense* and *C. acutatum* complexes have been related to avocado anthracnose (Jayawardena et al., 2016) and are reported as the main production constraint in the bigger avocado producer countries including Mexico (Silva-Rojas and Ávila-Quezada, 2011; Velázquez-del Valle et al., 2016) and Colombia (Gil and Morales-Osorio, 2019). *Colletotrichum* isolates are able to colonize inflorescences, leaves, stems, and fruits of avocado and typically generate semicircular or angular necrotic, sunken and wet-looking lesions (Cannon et al., 2012; Pérez-Jiménez, 2008). These injuries cause the fruits to be unsuitable for export, thus losing their commercial value.

In Colombia, the information regarding Ggg, *M. oryzae, P. cinnamomi,* and *Colletotrichum* distribution in Tolima for the past years is insufficient, even when previous reports from this and other zones in the country showed the high incidence of these pathogens in rice or avocado crops (Echeverri-Rico, 2016; Grisales et al., 2016; Prado-Patiño, 2016; Ramírez and Morales-Osorio, 2013). Moreover, in the case of Ggg, *Colletotrichum* and *P. cinnamomi* the diagnosis is currently done based on symptoms that could be confused with other diseases. For example, some symptoms caused by *P. cinnamomi* on root trees could be confused with the symptoms caused by *Cylindrocarpon destructans* infection (Ramírez-Gil, 2018), root asphyxiation caused by water

and temperature stress (Nakova, 2010; Ramírez-Gil, 2018) or tree poor nutrition (Akinsanmi et al., 2016). The misdiagnosis could lead to the implementation of inefficient management strategies increasing the negative effects on crop productivity.

In this context, the differential diagnosis of the diseases caused by these phytopathogens should rely on pathogen isolation and identification. For this reason, the present research aims to do an exploratory study of the presence of these four microorganisms in rice and avocado crops on the centrum of Tolima department. This information is going to be used as initial data for a second phase study that seeks to obtain epidemiological information about the prevalence of Ggg, *M. oryzae*, *P. cinnamomi* and *Colletotrichum* in Colombia.

MATERIALS AND METHODS

Rice and avocado sampling

In order to isolate and identify the fungal phytopathogen present in symptomatic rice and avocado plants with a preliminary diagnosis of crown sheath rot, rice blast, anthracnose and root rot were obtained from 9 and 21 different rice and avocado crops respectively located at the centrum zone of Tolima department (Supplementary Table 1). For rice samples, a presumptive diagnosis was done evaluating the symptoms and severity of crown sheath rot (Supplementary Table 2) and rice blast (Supplementary Table 3) (Ghazanfar et al., 2009). Plants with severity index higher than 2 for Ggg isolation or 4 for *M. oryzae* isolation were taken out of the field and transported at 4°C to the laboratory for pathogen isolation.

For avocado samples, necrotic roots of trees with symptoms of root rot were obtained. For this purpose, at least 10 trees per location were evaluated and classified according to the scale proposed by Ramirez-Gil (Ramírez-Gil, 2018) (Supplementary Table 3). Trees with symptoms recorded in the scale as 3 to 5 were used for roots sampling. Only secondary roots with initial symptoms of necrosis were selected and transferred at 4°C for pathogen isolation. On the other hand, 5 Avocado fruits (cv Hass) with initial anthracnose symptoms (lesion of 1 - 6 cm diameter), advance necrosis (lesions with > 6 cm diameter), modified fruits and healthy fruits were randomly selected from each evaluated location. The fruits were put into plastic bags and maintained refrigerated (4°C) until processing.

Isolation and purification of rice pathogens

Rice samples included sheath, culms and leaves of three different cultivars (F67, F68 and F2000). The sheath and culm samples were microscopically inspected to identify the presence of black-brown mycelium, perithecium and or hyphopodia suggestive of Ggg. Positive samples were cut into pieces of 5 cm^2 and transferred to moist chambers. To favour mycelium growth and perithecium opening for discharge of ascospores, the moist chambers were incubated at 25°C for 7 days. Then, the growing mycelium was aseptically transferred to PDA medium supplemented with chloramphenicol (50 µg/ml). Additionally, the remnant plant tissue was surface sterilized in sodium hypochlorite (3%) and ethanol (70%) for 1 min and washed with sterile water (3 min). Once sterilized, the treated plant tissue was transferred to PDA medium

and incubated at 25°C for seven days.

For *M. oryzae* or *Magnaporthe*-like fungi isolation, a similar approach was followed using rice leaves samples. The material was inspected looking for diamond-shape lesions. Positive samples were cut in pieces (5 cm^2) and transferred aseptically to moist chambers. As with Ggg samples, the moist chambers were incubated for seven days at 25°C and the growing mycelium was then transferred to Chloramphenicol supplemented PDA and further incubated at 25°C.

Individual colonies were purified on PDA for Ggg like isolates or Tomato-oatmeal medium (Oatmeal 20 g/L, tomato paste 20 g/L, bacteriological agar 15 g/L pH 7.0) for *M. oryzae* like isolates (Supplementary Table 4). Macroscopic and microscopic characteristics of pure colonies were observed and only isolates with phenotypic characteristics similar to those of the fungi of interest were preserved for molecular identification.

Isolation and purification of avocado pathogens

Necrotic secondary roots of avocado for the isolation of *P. cinnamomi* were initially washed with tap water for 5 min. Then the roots were dried at room temperature and the external cover of the root was carefully removed. All the roots were inspected to identify zones with partial necrosis. Once identified, the roots were superficially sterilized following the same protocol as mentioned for rice foliar tissue. The surface-sterilized roots were then transferred to PDA and V8-ACBPHR medium (Ramírez and Morales-Osorio, 2013) and incubated at 22°C in darkness for 7 days. All the growing fungi were further purified and preserved for phenotypic and molecular characterization. For *Colletotrichum* isolation, avocado fruits were inspected and the tissue with necrotic lessons was cut in pieces of 0.25 cm^2. These pieces were surface-sterilized (same protocol as described above) and transferred to PDA supplement with chloramphenicol (50 µg/ml). The agar plates were incubated at 25°C for five days. The fungi growth was verified, and the individual colonies were purified on new PDA medium. Only those colonies with growth, pigmentation and microscopic characteristics typical of *Colletotrichum* species were preserved for molecular characterization.

DNA extraction and molecular identification

For molecular characterization, Genomic DNA of each fungal strain was obtained using a modified protocol of CTAB (Cetyltrimethyl Ammonium Bromide) / Chloroform-isoamyl alcohol extraction described by Clarke (Clarke, 2009). Molecular characterization was done through ITS region sequencing using the universal primers ITS1 (5´- TCCGTAGGTGAACCTGCGG-3´) and ITS4 (5´- TCCTCCGCTTATTGATATGC-3´) (White et al., 1990). Polymerase chain reaction (PCR) amplification was carried out as described by Mosca et al. (2014). The thermal cycling profile of initial denaturation at 95°C for 3 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s and elongation at 72°C for 45 s were used (Mosca et al., 2014). The obtained amplimer was sequenced (Macrogen, Korea) and the ITS sequence obtained was compared with the corresponding reference data retrieved from GenBank database.

Sequence alignment was carried out using MUSCLE algorithm and adjusted manually. Phylogenetic analysis of the partial ITS sequences was carried out using the Maximum likelihood method or Neighbor-joining method with MEGA7 software (Kumar et al., 2016). Evolutionary distance matrices were generated by either Juker-Cantor, Tamura-Nei or Kimura 2-parameter method. The stability of tree topologies was accessed by bootstrap analysis based on 1000 resampling.
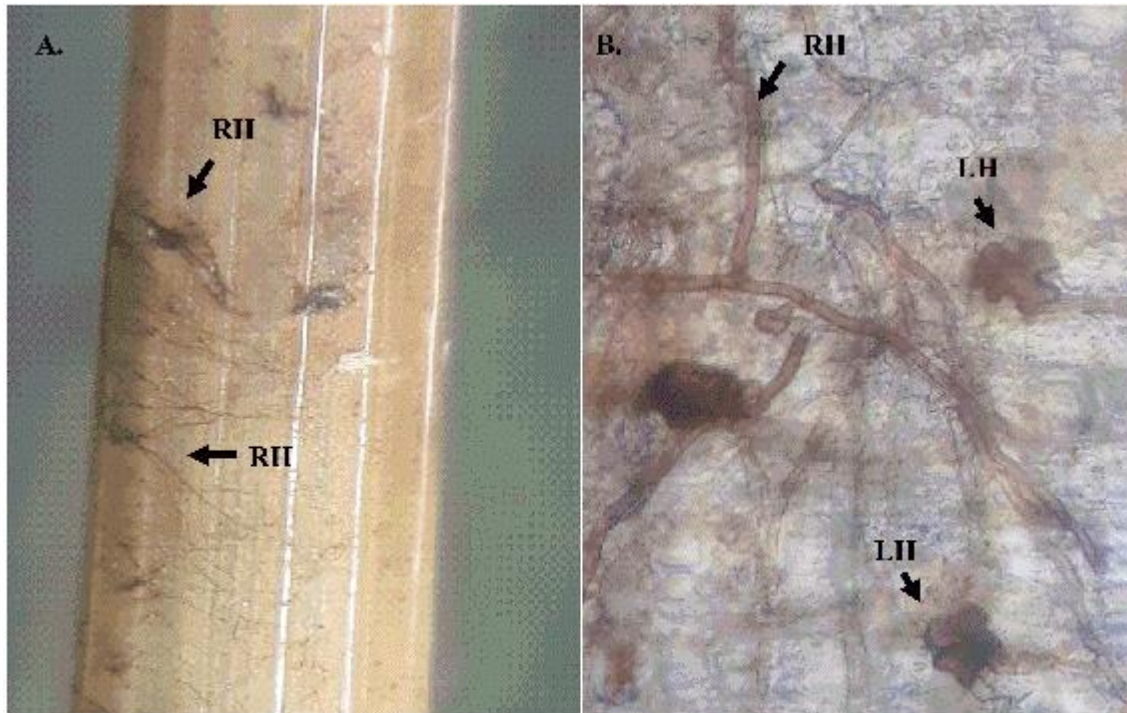
Figure 1. Symptoms of crown sheath rot and presumptive isolation of Ggg and other *Gaeumannomyces* isolates from rice samples.

RESULTS AND DISCUSSION

Phytopathogenic fungi obtained from rice samples

In order to contribute to the diagnosis of rice diseases in Tolima, in the present research, 53 samples of rice plants from 12 different crops located in the centrum and west part of the department (Supplementary Table 1) were evaluated and used for fungal pathogen isolation. As shown in Figure 1, the studied samples present brown septate hyphae and lobulated hyphopodia. Runner hyphae are evident macroscopically as black filaments growing on the surface of rice culms and are used as a preliminary diagnostic symptom of crown sheath rot. In sheath and culm tissues, the presence of these structures can be a sign of advanced Ggg colonization (Hawerroth et al., 2017; Hernández-Restrepo et al., 2016).

Figure 1 shows the runner hyphae (A) and lobulated hyphopodia (B) present on rice sheaths. Previous reports from FEDEARROZ diagnostic group have indicated the presence of Ggg in the south of Tolima and other departments of Colombia (Echeverri-Rico, 2016). However, other zones of Tolima have not been studied so far and are having problems of misdiagnosis due to the presence of unspecific symptoms and co-occurrence of fundal diseases in the same phenological stage. In the present study, 18 different isolates were obtained from the evaluated samples and presented the typical grey-black mycelium on PDA medium characteristic of *Gaeumannomyces* isolates. Most of them were recovered from Ambalema (13 isolates), Espinal (2 isolates), Piedras (2 isolates) and Ibague (single isolate). The ITS sequences of these fungi allowed us to classify them into the *Gaeumannomyces genus.* Moreover, as seen in Figure 2, most of the isolates were closely related to *G. oryzinus* ang *G. graminis var. graminis* with percentages of similarity higher than 98 % (Supplementary Table 5).

The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.0701)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The tree was rooted with *M. oryzae* (CBS 255.38 and CBS 128304).

Originally, *G. oryzae* and Ggg were described as the same microorganism since there are able to infect rice plants; they do not have morphological differences and the ITS sequence by itself is not strong enough to separate them into different clades (Walker, 1972). However, recent phylogenetic studies done using the sequences of several housekeeping genes such as ITS, LSU, *rpb1* and *tef1* demonstrated that both microorganisms correspond to different clades within the
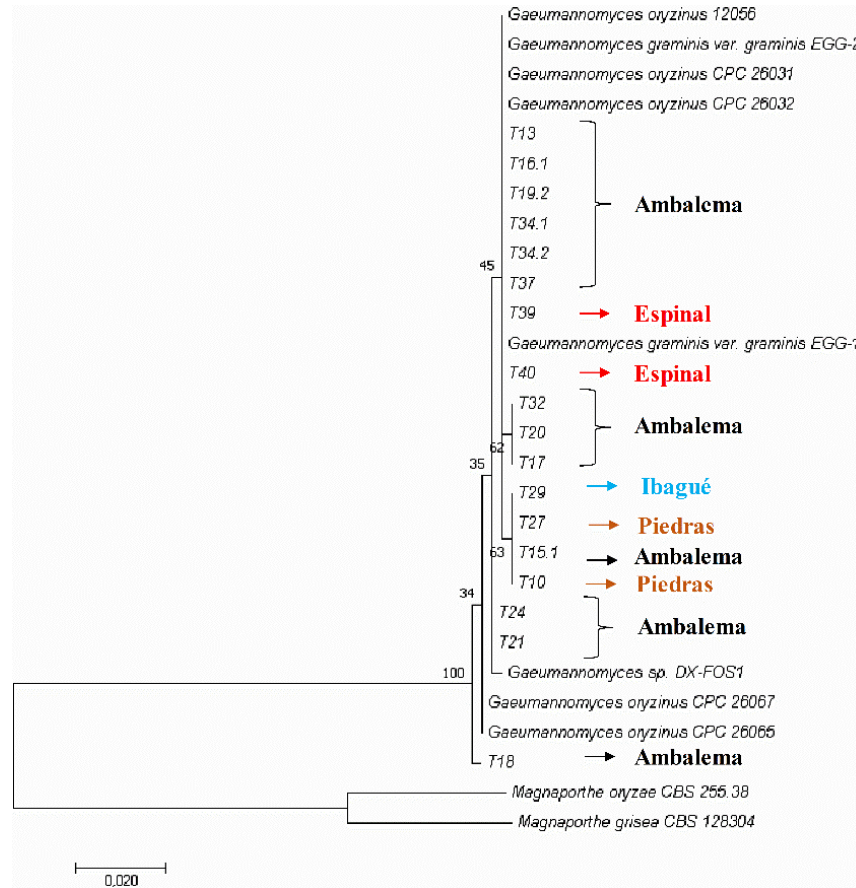
Figure 2. Molecular phylogenetic analysis by maximum likelihood method of partial ITS1 sequences of *Gaeumannomyces* isolates.

Gaeumannomyces genera (Hernández-Restrepo et al., 2016). Ggg and *G. oryzae* are commonly isolated from rice cultivars but only Ggg has been recognized as the causal agent of crown sheath rot. Further information is needed in order to confirm the pathogenesis of *G. oryzae* and its relationship with crown sheath rot.

On the other hand, as can be seen in Figure 3 six isolates of rice blast lesions were purified. All of them correspond to fungi of the *Magnaporthe* genera. Based on the ITS sequences, it can be suggested that they belong to the *M. oryzae* species. These fungi were isolated from samples coming from Espinal, Piedras and Ibague. In other locations, isolates of the *Curvularia* and *Fusarium* genera were identified based on phenotypic characteristics, but they were not identified by ITS sequencing (Data not shown).

The evolutionary history was inferred by using the Neighbor–joining method based on the Tamura-nei model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The tree was rooted with *Barretomyces calatheae* (CBS 129274). The

similarity of *M. oryzae* with other species such as *M. grisea* is explained by the close relationship of both species. *M. grisea* and *M. oryzae* belong to the *M grisea complex* (Zhang et al., 2016). They are morphologically indistinct but could be differentiated from each other based on its host plant (rice for *M. oryzae* and grasses for *M. grisea*) and multi Locus Phylogenetic analysis using conserved genes like actin, beta-tubulin, and calmodulin (Couch and Kohn, 2002; Klaubauf et al., 2014). Although *M. oryzae* is the currently accepted causal agent of rice blast, some reports indicate cross-infection in rice with *M grisea* isolates (Choi et al., 2013). Therefore, the presence of both pathogens should be monitored to prevent rice blast outbreaks and cross-infection with grasses.

***Colletotrichum* sp. isolates as the causal agent of anthracnose disease on avocado Hass fruits in Tolima**

In the present research, 21 different avocado crops in Tolima were sampled in order to determine the presence
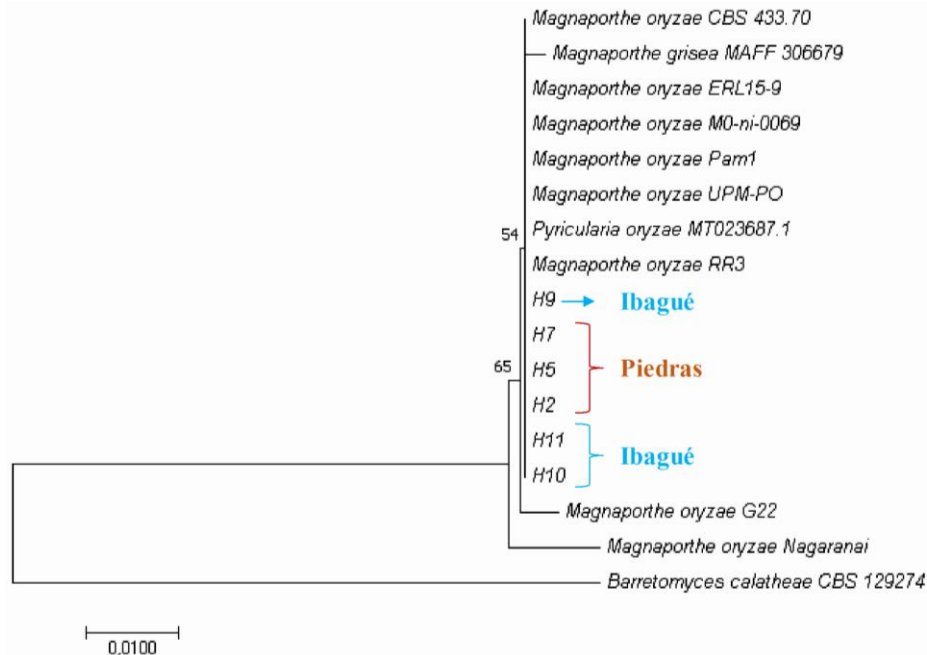
Figure 3. Molecular Phylogenetic analysis by Neighbor-joining method of partial ITS1 sequences of *Magnaporthe* isolates.

of *Colletotrichum* as causal agent of anthracnose symptoms observed on fruits in the evaluated crops. 13 *Colletotrichum*-like fungi were isolated from avocado fruits (Hass cv.) with necrotic lesion (Figure 4A). From them, only those isolates that presented orange-black pigmentation and abundant production of hyaline septate ovoid conidium were preserved for identification (Figure 4B-C). Figure 4 shows the typical circular necrotic wound in Avocado cv Hass (A) and the macroscopic (B) and microscopic (C) characteristics of isolate F27 isolated from this kind of lesion.

Colletotrichum is a widespread Ascomycota fungus that has been reported as plant pathogen in economically important fruits crops such as avocado. Due to its distribution and incidence this fungus is classified as one of the ten most important fungal phytopathogens of the world causing losses in production up to 50% in several fruits and vegetable crops (Dean et al., 2012). Related to its impact on crops, the isolation and identification of *Colletotrichum* species have been a major field of research for the last decades. The taxonomy of *Colletotrichum* is complex. Currently, 11 fugal complexes have been reported for this genus (Jayawardena et al., 2016). From them, species belonging to the *C. gloeosporioides, C. acutatum* and *C. boninense* complexes are the most associated with anthracnose on avocado fruits.

In Colombia, some efforts have been done in order to identify the prevalence and distribution of these pathogens in avocado (Cobo-Nuñez, 2017; Grisales et

al., 2016) and others crops such as tomato and mango (Cabrera et al., 2018). As expected, different members of the *C. gloeosporioides* and *C. acutatum* were reported for avocado crops in Antioquia. According to their ITS sequences, two of the 13 isolates recovered in the present study, F3 and F28, were closely related to *C. acutatum* complex and were isolated from two different locations, "La Luisa" and "Pan de azúcar" respectively. The rest of the purified *Colletotrichum* fungi belong to the *C. gloeosporioides* complex and were related with several species including *C. gloeosporioides, C. siamense* and *C. fruticola* (Figure 5, Supplementary Table 6). *C. siamense* and *C. fruticola* have been previously reported as the causal agent of Anthracnose and Soft Rot in Avocado Fruits cv. Hass in Mexico and Australia (Fuentes-Aragón et al., 2018; Giblin et al., 2018) but to date, they have not been reported in Colombia. The isolates F27, F31 and F33 were isolated from the same location (La Luisa) but in different farms and were closely related to *C. gloeosporioides* and *C. boninense* another *Colletotrichum* species associated with anthracnose on avocado.

Other fungi obtained were *Pestalotiopsis sp.* F36 and *Alternaria* sp. F36.2 both of them from samples collected at "La cerrajosa". *Pestalotiopsis microspora* has been previously isolated from anthracnose lesion in avocado in Kenya (Kimaru et al., 2018) and *P. clavispora* in Chile (Valencia et al., 2011). In this context, and since no *Colletotrichum* fungi were obtained from this location, it is possible to suggest that *Pestalotiopsis* sp. F36 is the
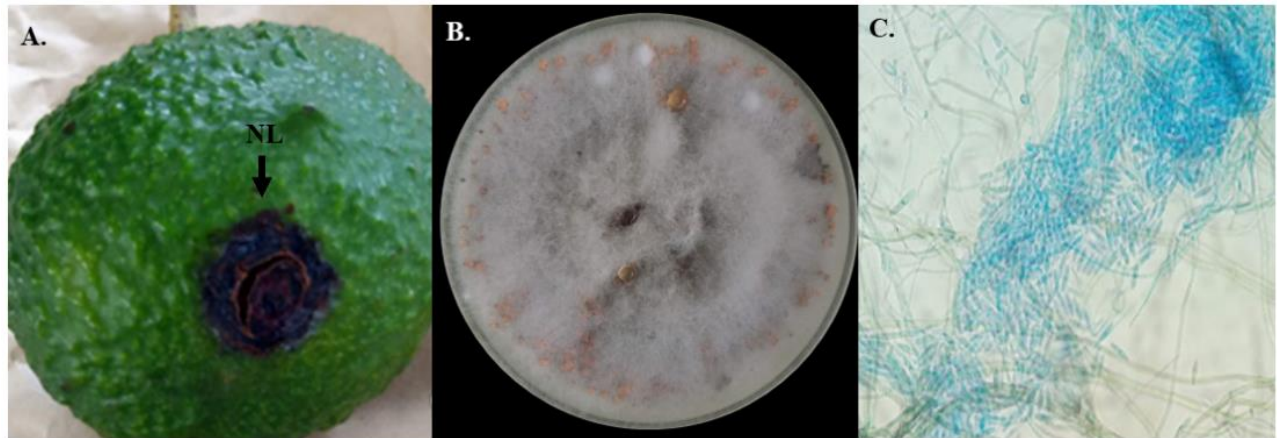
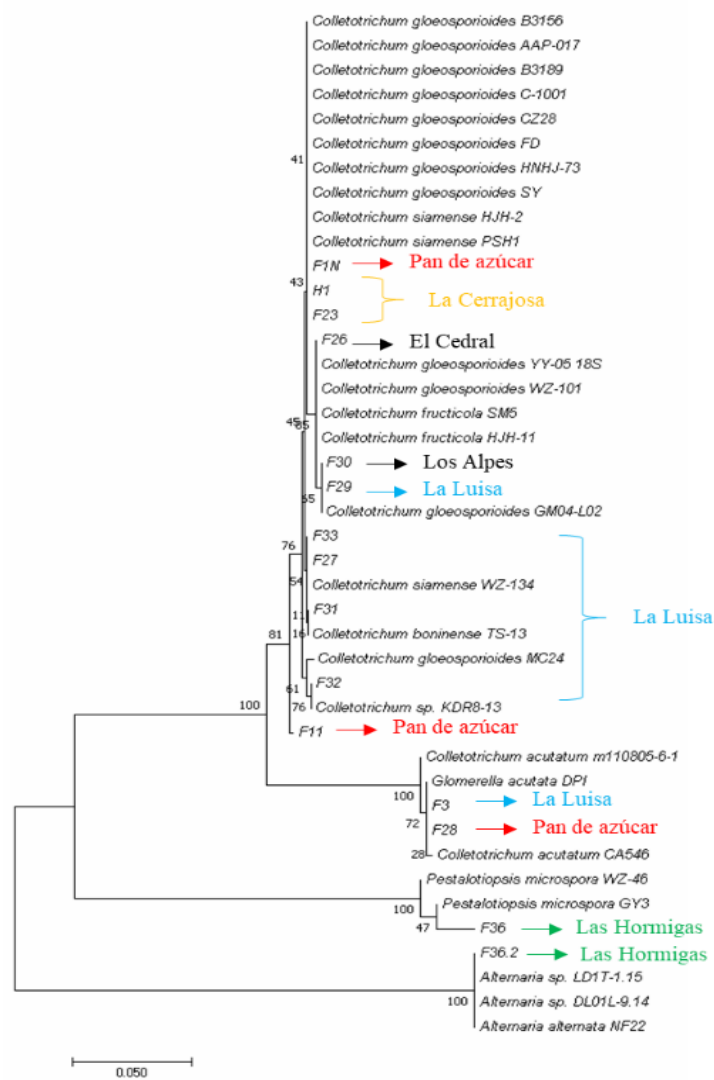**Figure 4.** Phenotypic characteristics of *Colletotrichum* sp. F27.

Figure 5. Molecular Phylogenetic analysis by Neighbor-joining method of partial ITS1 sequences of *Colletotrichum* and other isolates obtained from avocado fruits.
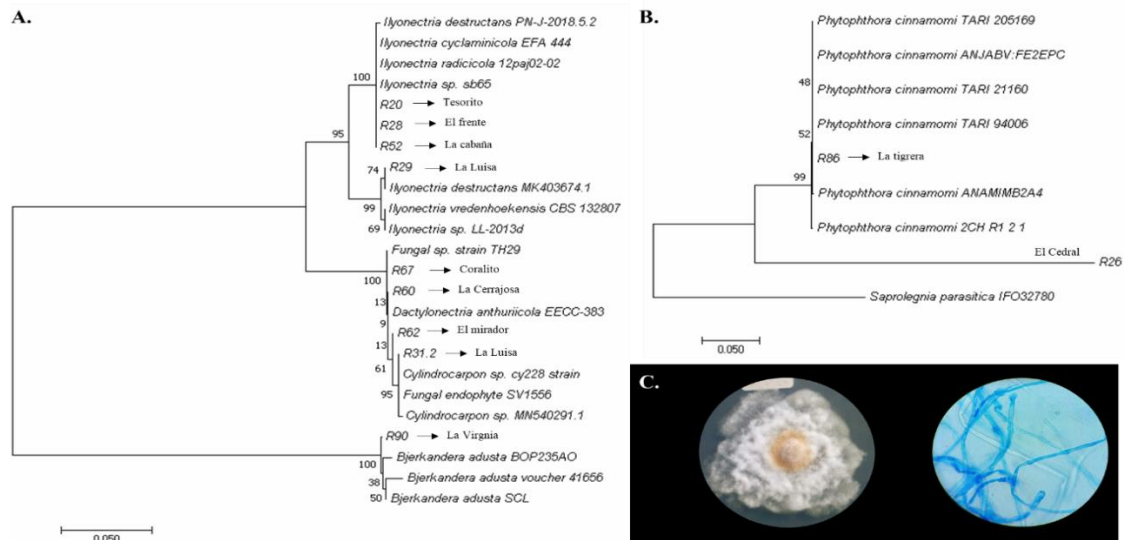
Figure 6. Molecular phylogenetic analysis by Neighbor joining method of partial ITS1 sequences of *Ilyonectria sp., Phytophthora cinnamomi* and related fungi obtained from avocado roots. (A) *Ilyonectria* destructans and related fungi. (B) *Phytophthora cinnamomi* isolates.

causal agent of the anthracnose symptoms observed in that location.

The evolutionary history was inferred by using the Neighbor–joining method based on the Tamura-nei model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. As previously mentioned, ITS alone sequence is not enough to differentiate *Colletotrichum* species (Damm et al., 2012). For that reason, it is necessary to complement the data obtained with a more complete genetic study in order to bring a clear conclusion about the possible new reports of *C. fruticola* and *C. siamense* affecting avocado crops in Colombia. With the obtained data, it is only possible to conclude the presence of several isolates belonging to the *C. gloeosporioides* and *C. acutatum* complex in Tolima.

Phytopathogenic fungi isolated from necrotic avocado roots

Root rot is one of the most devastating diseases in avocado crops around the world. This disease is generated by the oomycete *P. cinnamomi* (Hardham and Blackman, 2018). The infection with this phytopathogen causes root necrosis, wilting, leaf loss and reductions in fruit development and production (Granada et al., 2020). In Colombia, the incidence of *P. cinnamomi* has been documented in specific zones such as Antioquia (Ramírez-Gil, 2018, 2017), Bolivar and Sucre (ICA, 2012). In order to complement the available information about the distribution of this pathogen, a targeted

isolation procedure was developed using samples obtained from trees with root rot symptoms in Tolima.

103 samples were processed from 21 avocado crops located at 15 different points of the central zone of Tolima. From them, eleven phytopathogens were recovered (Figure 6). Eight isolates correspond to fungi belonging to the genera *Ilyonectria* (anamorph. *Cylindrocarpon*) and were closely related to known phytopathogens of avocado like *I. destructans* that were previously isolated in Colombia as the causal agent of black root rot disease in Antioquia avocado plantations (Ramírez and Morales, 2013).

For A and B trees, the evolutionary history was inferred by using the Neighbor–joining method based on the Tamura-nei model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. In B, the tree was rooted with *Saprolegnia parasitica* IFO32780. (C) Macroscopic and microscopic characteristics of *P. cinnamomi* R86.

Only two isolates of *Phytophthora* genera, R26 and R86, were isolated from secondary roots with low necrosis degree (Figure 6B and C, Supplementary Table 6). Both microorganisms are closely related to *P. cinnamomi* and were obtained from "El cedral" and "La tigrera" avocado plantations respectively that have early symptoms of the disease. One of the main difficulties in the isolation of *P. cinnamomi* is its low growth rate under *in vitro* conditions. This causes that other saprophytic microorganisms with higher growth rates reduce or inhibit *P. cinnamomi* growth. The use of culture media with complex mixtures of antibiotics does not always work as it could be seen in this work. Here, from the presumptive

P. cinnamomi samples, a high number of saprophytic microorganisms were isolated including several species of *Fusarium, Acremonium, Verticillium* and *Phytium*. The presence of these fungi was also related to the necrotic state of some samples which had evident secondary infection with bacterial and fungi that prevented *P. cinnamomi* isolation. A more careful selection of the samples should be done in future works in order to improve the recovery rate of *P. cinnamomi*.

It is important to emphasize that despite the low isolation rate of pathogens recovered from avocado roots in this work, *Ilyonectria* and *Phytophthora* present similar symptoms. For this reason, both diseases can be diagnosed incorrectly, which can affect the crop phytosanitary management. To avoid this, it is important to establish programs that include follow-up processes for the phytopathogens present in susceptible crops so that an appropriate and early diagnosis can be made to improve crop productivity.

Conclusion

Major diseases of Tolima's rice crops are related to the rice blast and crown sheath rot diseases. In this study the presence of different pathogens related to these diseases, *G. graminis, G. oryzinus* and *M. oryzae* (sin= *P. oryzae*) was confirmed, being the first formal report of these fungi in different regions of Tolima. Similarly, the presence of isolates belonging to *C. gloeosporioides* and *C. acutatum* complex on avocado fruits supports the hypothesis of these fungi being the causal agent of anthracnose in the region. More accurate molecular identification and field re-inoculation studies should be done to unequivocally establish the role of these pathogens on rice and avocado fruit diseases. Finally, since more than one possible phytopathogen was isolated from root tissues, further studies are needed to confirm the role of both Ilyonectria (Anam= Cylindrocarpon) and *Phytophthora* isolates on the root rot syndrome observed on avocado trees. This information should be used in order to improve the management strategies for these pathogens on fields aiming to increase avocado production in Tolima. Currently, the microorganisms obtained from this study are being used for biocontrol and chemical tests to find an adequate method for their control on the field and are going to be identified to species level using a MLST approach.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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SUPPLEMENTARY TABLES

Supplementary Table 1. Severity scale used for rice plants with crown sheath symptoms.

Severity	Symptoms
0	No lesion observed
1	Incipient lesions of dark color in the leaf sheath only. Slightly diseased material.
2	Significant necrosis of sheath with presence of perithecia
3	Presence of dark mycelium accompanied by hyphopodia, generally between the outer side of the stem and the leaf sheath.
4	Wilting of the stem due to mycelial growth of the fungus
5	Generalized drying of the stem and leaves. Tissue death

Supplementary Table 2. Severity scale used for rice plants with rice blast symptoms.

Severity	Foliar (aerial) symptoms
0	No lesion observed
1	Small brown specks of pin-point size
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin. Lesions are mostly found on the lower leaves
3	Lesion type same as in 2, but significant number of lesions on the upper leaves
4	Typical susceptible blast lesions, 3 mm or longer infecting less than 4% of leaf area
5	Typical susceptible blast lesions of 3mm or longer infecting 4-10% of the leaf area
6	Typical susceptible blast lesions of 3 mm or longer infecting 11-25% of the leaf area
7	Typical susceptible blast lesions of 3 mm or longer infecting 26-50% of the leaf area
8	Typical susceptible blast lesions of 3 mm or longer infecting 51-75% of the leaf area many leaves are dead
9	Typical susceptible blast lesions of 3 mm or longer infecting more than 75% leaf area affected

Source: Ghazanfar et al. (2009).

Supplementary Table 3. Severity scale used for avocado plants with root knot symptoms.

Severity	Foliar (aerial) symptoms	Root symptoms
0	Healthy plants with abundant dark green foliage and actively growing leaf buds. No symptoms of illness.	>90% of viable secondary roots
1	Slight yellowing of the leaves and lack of active shoots leading to stunned growth.	Symptomatic (necrotic) roots 10-15%
2	Pronounced chlorosis on leaves and growth arrest.	Symptomatic (necrotic) roots 16-25%
3	Generalized chlorosis leaf, wilt and slight defoliation <35%.	Symptomatic (necrotic) roots 26-50%
4	Generalized chlorosis of leaves, wilting and defoliation between 35.1-90%.	Symptomatic (necrotic) roots 50-90%
5	Descendant death and severe defoliation> 90%.	Symptomatic (necrotic) roots >90%

Source: Ramírez-Gil (2018).

Supplementary Table 4. Closest neighbors of rice isolates.

Isolate	T10	T13	T15.1	T16.1	T17	T18	T19.2	T20	T21	T24	T27	T29	T32	T34.1	T34.2	T37	T39	T40
ITS sequence length (pb)	532	559	486	558	495	554	495	554	497	547	556	546	571	556	526	571	528	516
Closest neighbor (% similarity)																		
G. oryzinus CPC26067	99.25	98.57		98.75	99.19	99.1		98.82	98.79	98.9	98.74	98.9	98.72	98.5	98.5		98.72	
G. oryzinus CPC26032	99.81	98.57	99.59	99.44		99.26	99.39		98.79	98.7	99.44	99.62	99.26	99.06	99.06	98.81	99.26	99.19
G. oryzinus CPC12056	99.81		99.59		99.79		99.39	99.44			99.44	99.62	99.26	99.06	99.06	98.81	99.26	99.19
G. oryzinus CPC26065				98.75		99.1		98.92		98.9		98.9						
G. oryzinus CPC26031			99.59															99.19
Gaeumannomyces sp. DX-FOS1									99.0									
G. graminis var graminis EGG-1							99.6										99.01	
G. graminis var graminis EGG-2		99.81		99.81	99.4							99.44	99.44					

The closest microorganisms were retrieved from the Genbank database.

Supplementary Table 5. Closest neighbors of rice isolate %similarity.

Isolate	H2	H5	H7	H9	H10	H11
ITS sequence length (pb)	543	544	544	544	466	487
Closest neighbor (% similarity)						
M. oryzae CBS433.70	99.45	99.63	99.81	99.25		
M. oryzae ERL 15-9	99.45		99.81			99.17
M. oryzae UPM-PO	99.63	99.63	99.81	99.43		
M. oryzae Nararanai		99.62		99.07		
M. oryzae G22		99.26				
M. oryzae Mo-ni-0669					100	99.79
M. oryzae Pam 1					99.57	99.39
M. oryzae RR3						
M. grisea MAFF306679		99.63	99.45			
M. grisea 70-15				99.07	99.57	

Supplementary Table 6. Closest neighbors of avocado isolates.

Isolate	H1	F1N	F3	F11	F23	F26	F27	F28	F29	F30	F31	F32	F33	F36.2
ITS sequence length (pb)	511	554	576	491	506	578	551	587	554	515	507	565	564	551
Closest neighbor (% similarity)														
Glomerella sp. ERS054	100				100									
Glomerella sp. ERS043					100									
Glomerella acutata DPI			99.65						99.83					
Colletotrichum sp. KDRS-13												99.11		
Colletotrichum gloeosporioides AAP-17	100				100									
Colletotrichum gloeosporioides B3189	100	100												
Colletotrichum gloeosporioides B3186		100												
Colletotrichum gloeosporioides SY		100		98.27										
Colletotrichum gloeosporioides FD				98.27										
Colletotrichum gloeosporioides HNHJ-73				98.27										
Colletotrichum gloeosporioides LCM 984.01							100							
Colletotrichum gloeosporioides cb-2							100							
Colletotrichum gloeosporioides CZ28												98.76	99.12	
Colletotrichum gloeosporioides MC24												98.76		
Colletotrichum gloeosporioides B3156											99.4			
Colletotrichum gloeosporioides C1 001											99.4			
Colletotrichum gloeosporioides GM04-L02										100				
Colletotrichum gloeosporioides MKC5											99.81			
Colletotrichum gloeosporioides YY-05						99.86			99.82		99.81			
Colletotrichum gloeosporioides WZ-101						98.96			99.82					
Colletotrichum acutatum A3						99.14			99.82					
Colletotrichum acutatum ml 10805-6			99.48					99.66						
Colletotrichum acutatum CA546			99.48					99.66						
Colletotrichum fructicola HJH-11									99.82					
Colletotrichum fructicola CBS 125390									99.82					
Colletotrichum fructicola SM40						98.97								
Colletotrichum fructicola 5						98.97								
Colletotrichum siamense WZ-134													99.29	
Colletotrichum siamense HJ-2													99.29	
Colletotrichum siamense PSH1											99.4			
Colletotrichum boninense TS13							100							
Alternaria sp. DL01L-9														100
Alternaria sp. LDL1T-1.15														100
Alternaria alternata NF22														100

ISOLATE	R20	R26	R28	R29	R52	R31.2	R60	R62	R67	R86	R90
ITS sequence length (pb)	522	882	545	539	519	569	572	478	523	890	632

Supplementary Table 6. Contd.

Closest neighbor (% similarity)										
Phytophthora cinnamomi TARi 94006									100	
Phytophthora cinnamomi TARi 205169									100	
Phytophthora cinnamomi TARi 21160									100	
Phytophthora cinnamomi ANAMIMB 2A4		93.96								
Phytophthora cinnamomi ANJABV_FE2EPC		93.96								
Phytophthora cinnamomi 2CH_R1_2_1		94.23								
Ilyonectria sp. sb65	100		99.81		100					
Ilyonectria sp. LL-2013d				99.25						
Ilyonectria cyclaminicola EFA 444	100		99.08							
Ilyonectria destructans PN-J-2018.5.2	99.81		99.26		99.81					
Ilyonectria vredenhoekensis CBS132807				99.25						
Ilyonectria radicola 12paj02-02					100					
Cylindrocarpon sp.						98.72				
Cylindrocarpon cy228						99.08	98.88	99.16	99.04	
Cylindrocarpon RGO_LsH_C2								99.58		
SV1556						99.81	98.5			
TH29							99.27	100	100	
Dactylonectria anthuriicola EECC-383									100	
Bkerkandera adusta SCL										99.37
Bkerkandera adusta BOP235AO										99.34
Bkerkandera adusta 06101_7A										99.66

The closest microorganisms were retrieved from the Genbank database.