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Avocado wilt complex disease, implications and management in Colombia

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Complejo marchitez del aguacate, implicaciones y manejo en Colombia

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ABSTRACT

Keywords:
Biotic and abiotic causal agents
Economic impact
Management practices

The avocado crop is widely grown in Colombia, especially the commercial variety called Hass. This accelerated cultivation of avocado has not been properly planned locally, but influenced by external recommendations, which in many cases are not applicable to the local conditions in Colombia. This situation has led to the failure of many avocado crops, which are faced with serious technological lags, inducing low sustainable levels. Amongst the most serious limitations is the avocado wilt complex (AWC). This term is used to describe a multi-complex diseases associated with different causal agents, which can be of biotic and abiotic origins, and can affect roots and stem of avocado plants in all stages of development, causing similar aerial symptoms. The objective of this study was to describe the AWC and its implications in Colombia, emphasizing essential aspects such as symptomology, causal agents, incidence, distribution, economic impact, associated problems, factors predisposing, management practices, technology of information, and future scenarios. The information described in this manuscript is part of multiple research developed in the field, greenhouse and laboratory by the author for more than 8 years, in addition to the literature reported in Colombia in the different topics described above. This study should serve as a fundamental basis for understanding the impact of AWC and give the basic information for a correct management technically and scientifically.

RESUMEN

Palabras clave:
Agentes causales bióticos y abióticos
Importancia económica
Prácticas de manejo

El cultivo de aguacate en Colombia viene creciendo en los últimos, especialmente variedades comerciales como el Hass. La acelerada siembra de aguacate ha carecido de una planificación adecuada y se ha desarrollado bajo recomendaciones técnicas externas, las cuales en muchas circunstancias carecen de aplicabilidad para las condiciones del país. Esta situación ha conducido al fracaso de muchas explotaciones, donde existen fuertes rezagos tecnológicos que evitan que este sistema productivo sea sostenible. Dentro de los mayores limitantes se encuentra el complejo marchitez del aguacate (CMA), termino definido para múltiples patologías asociadas a distintos agentes causales, los cuales pueden ser de origen biótico o abiótico, que afectan el sistema de raíces y la base del tallo de plantas de aguacate en todos los estados de desarrollo, induciendo síntomas de expresión similares en la parte aérea. El objetivo de este trabajo fue realizar una descripción del CMA en Colombia, realizando énfasis en aspectos básicos como sintomatología, agentes causales implicados, incidencia, distribución, importancia económica, problemática asociada, factores determinantes, prácticas de manejo, uso de tecnologías de la información y escenarios futuros. La información que se describe hace parte de múltiples ensayos investigativos desarrollados en campo, invernadero y laboratorio por el autor por un periodo de tiempo de más de 8 años, además de la literatura reportada para Colombia en los distintos tópicos descritos anteriormente. Este trabajo se convierte en la base fundamental para entender la importancia del CMA y sienta las bases para definir la manera de abordarlo de manera técnica y científica.

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Avocado (*Persea americana* Mill.) is grown in 59 countries. These include, both tropical and subtropical countries, but the crop's commercial production is greatest in the Americas, where it serves as an engine of rural development in countries such as Mexico, Chile, Dominican Republic, United States (California and Florida), Colombia, Peru, Ecuador, Brazil, among others (FAO, 2017; Ramírez-Gil, 2017a; Bernal and Díaz, 2014). In Colombia, this crop is currently distributed in 18 departments, where Bolivar, Tolima and Antioquia, are the most important in for cultivation. The majority of these are native species that have not undergone any process of technical development, and their production are principally for the local market (Ramírez-Gil, 2017a; Bernal and Díaz, 2014). This situation has changed in recent years, thanks to the technical development of commercial varieties, of which Hass is the most prominent. This occurs particularly in moderately cold climates, and has been marked by an increase in *per capita* consumption in Colombia and improved export potential, both in its fresh and processed forms (Ramírez-Gil *et al*., 2018; Ramírez-Gil, 2017a; Agronet, 2016; Bernal and Díaz, 2014).

The most important limitations for avocado production are diseases. Diseases significantly impact avocado growth and productivity, as well as increase cost of production for farmers if they have to control outbreaks (Ramírez-Gil *et al*., 2017a). Of all avocado diseases, avocado wilt complex (AWC) stands out. AWC is a complex that causes multiple diseases associated with several biotic or abiotic origin causal agents, wherein each can induce similar or specific symptoms in their host (Ramírez-Gil *et al*., 2017a; Ramírez-Gil *et al*., 2014a). Oomycete *Phytophthora cinnam*omi Rands is considered the most significant causal agent in AWC, due to its frequency, aggressiveness, and the losses it causes (Ramírez-Gil *et al*., 2017a; Ramírez-Gil *et al*., 2014a; Tamayo, 2007; Zentmyer, 1984; Zentmyer, 1980). This does not imply the existence of other associated pathogenic agents with AWC such as *Phytophthora heveae* Thompson, *Phytophthora citricola* Sawada, *Verticillium* sp., *Armillaria mellea* (Vahl: Fr.) Kumm, *Armillaria* sp., *Cylindrocladium* sp., *Rosellinia* sp., *Fusarium solani* Sacc., *Fusarium oxysporum* Schlecht, *Fusarium equiseti* (Corda) Sacc. *sensu* Gordon, *Rhizoctonia* sp., *Phymatotrichum omnivorum* (Shear) Duggar, *Cylindrocladiella* sp., *Pythium* sp., *Pythium vexans, Cilindrocarpon destructans* and the nematodes *Helicotylenchus* sp., *Rotylenchulus* sp. and *Pratylenchus* sp. (Parkinson *et al*., 2017; Ramírez-Gil *et al*., 2017a; APS, 2017; Ramírez-Gil *et al*., 2014a; Ramírez-Gil and Morales, 2013; Vitale *et al*., 2012; Tamayo, 2007; Zentmyer, 1984; Zentmyer, 1980). In addition, other factors associated with abiotic origin have been reported; for example, hypoxia-anoxia, a major cause of death in avocado crop (Ramírez-Gil *et al*., 2017a; Ramírez-Gil *et al*., 2014a; Stolzy *et al*., 1967).

Data regarding the economic loss of AWC at a global level are scarce, and the vast majority of existing reports are associated with *P. cinnamomi,* with losses reported in commercial plantations of 45 to 90% (Tamayo, 2007; Zentmyer, 1980). Ogawa and Lyons (1983) reported that in California, United States, *P. cinnamomi* alone decreases the productivity of avocado between 20 and 25%. Its impact is such that Mora *et al*. (2007) estimated that 5% of the surface area planted with avocado in Mexico was infected with this pathogen, and that it was present in all of the producer municipalities. To quantify the economic effects of AWC, Ramírez-Gil *et al*. (2017a) proposed a new approach based on a study carried out for seven years covering all stages of development, under different production systems, and quantifying the losses in terms of mortality, reduction in production and management implications.

Regarding the management of AWC, there is no consensus in the results of known studies carried out for *P. cinnamomi*, which indicates the need for an integrated strategy. So far, strategies reported include, standing out the use of resistant rootstocks, antagonism with microorganisms, solarization, pruning, reduction of moisture in the soil, animal manure or compost, different types of mulch, fertilization rich in calcium, phosphorous and silicon, and application of fungicides in the soil and foliar of products such as potassium and aluminum phosphite, and metalaxyl, (Ramírez-Gil *et al*., 2017b; Richter *et al*., 2011; Messenger *et al*., 2000; Zentmyer, 1980; Broadbent and Baker, 1974).

Despite the importance of Hass avocado variety and the impact of AWC disease on the crop in Colombia, little is known about the interaction between pathogens, plants, environment, edaphoclimatic conditions, microbiota, and other organisms and agronomical management under specific conditions in each planted area. Integrated management strategies are not always globally applicable, therefore basic knowledge and technological tools should be developed for each specific place looking for a precision or site-specific agronomical management, which is considered to save resources, increase yields, decrease losses and in general, support crop sustainability. Based on the above, the objective of this manuscript was to formulate a description of AWC and its implications in Colombia, emphasizing on basic elements such as symptomology, associated causal agents, incidence, and distribution, economic impact, problematic of the disease, factors associated, management practices, and future scenarios. The information described in this manuscript is part of multiple studies developed in the field, greenhouse, and laboratory condition by the author for more than 8 years (2009-2017). In addition, to the literature reported in Colombia.

Avocado production system in Colombia

Based on different visits that the author has make to the avocado areas in Colombia, there are three basic production systems, (i) natives, (ii) Papelillos-Green, and (iii) Hass. Natives are characterized as natural systems, distributed throughout the all of Colombia, particularly in areas where they can be considered as "natural forests of avocado, because of its abundance. This distribution is a consequence of hybridization among races (*P. americana* var. *drymifolia, P. americana* var. *guatemalensis,* and *P. americana* var. *americana*) and has generated great variability, making avocadoes to now grow under diverse environmental conditions (Galindo-Tovar *et al*., 2008) (Figure 1). In these areas, few agronomic practices are performed. Systems ii and iii are associated with commercial varieties, generated from the races described above (Hass, Reed, Fuerte, Colin, Colinred, Lorena, Choquete, Booth, Semil, Trinidad, Santana, among others), which are planted in specific regions based on elevation, and edaphoclimatic condition. In these systems, there are different levels of technical inputs (Figure 1).

Origin and symptomatology of avocado wilt complex

Avocado wilt complex (AWC) is a term that has been used to describe multiple pathologies associated with distinct causal agents, which can be of biotic or abiotic origin, that affect roots, vascular system, and stem of avocadoes. These induce similar or specific symptoms in aerial part of the plants. AWC disease can occur in all stages of development of their host (Ramírez-Gil *et al*., 2017a; Ramírez-Gil *et al*., 2014a).

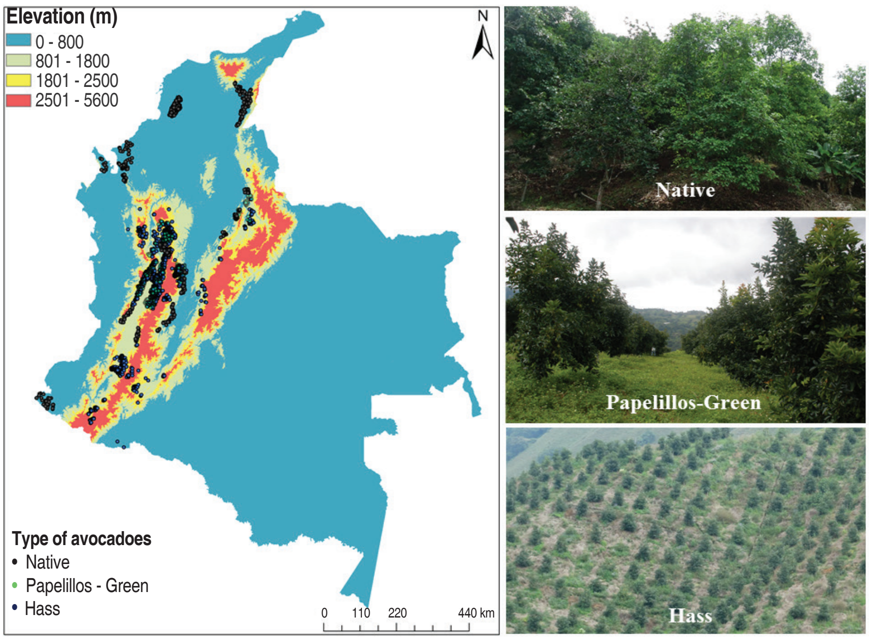

Figure 1. Localization and pictures of different avocadoes system in Colombia.

The main expression of AWC was characterized by foliar yellowing, tissue flaccidity, growth retardation, excessive flowering, and fructification of low quality in adult trees. In advanced stage, this can cause total defoliation, dieback, and plant death. In roots, the symptoms are associated with primary, secondary, and tertiary root rot. Other symptoms, which can be specific to different causal agents for AWC, are rapid leaf loss, hemi-lateral branch death, leaves can keep adhered to the plant, brown coloration in the vascular bundles, cankers at the base of the stem, pith rot, necrosis in the vascular bundles, among others. The visual symptoms and their causal agents are reported in the Figure 2.

Associated causal agents of avocado wilt complex

In different systematic sampling in Antioquia department, Colombia, a series of causal agents associated with the AWC have been reported. In these studies, a polyphasic approach for the identification of AWC disease caused by biotic or abiotic has been used, combining different parameters such as the detailed symptomatology description, isolation of microorganisms, morphological and molecular characterization of biotic causal agents, and pathogenicity test in avocado plants (Ramírez-Gil, 2018).

For the first work associated with AWC concept, seven causal agents were reported (Ramírez-Gil *et al*., 2014a), while for the second this number increased to 14 (Ramírez-Gil, 2018; Ramírez-Gil *et al*., 2017a). The causal agents involved were, *P. cinnamomi*, *P. citricola,*

Figure 2. General and specific symptoms associated with avocado wilt complex and their causal agents. A to F. General symptoms associated with AWC; G to L. Specific symptoms associated with AWC; A and B. Generalized yellowing and wilting of the leaves caused by *Phytophthora cinnamomi*; C and D. Excessive flowering, fructification of low quality in adult trees, defoliation, and dieback caused by hypoxia-anoxia and *Phytophthora cinnamomi* respectively; E and F. Secondary, tertiary and primary root rot caused by *Phytophthora cinnamomi* and *Phytophthora citricola* respectively; G. Wilting and leaf loss caused by hypoxia-anoxia; H. Hemilateral wilting and yellowing caused by *Verticillium* sp.; I. Pith rot caused by *Lasiodiplodia theobromae;* J. Cankers at the base of the stem by *Phytohpthora heveae;* K. Brown coloration in the vascular bundles caused by *Verticillium* sp.; L. Root atrophy.

P. heveae, P. palmivora, Verticillium albo-altrum, Verticilliun dahliae, Lasiodiplodia theobromae, C. destructans, Cylindrocarpon sp., *Rosellinia* sp., *Fusarium oxysporium sensu lato*, *Pythium cucurbitacearum*, *Phytopythium vexans*, *Pythium* sp., hypoxia-anoxia, and root atrophy. Each causal agent induced similar or particular symptoms associated with AWC, verified using pathogenicity tests (Ramírez-Gil, 2018; Ramírez-Gil *et al*., 2017a; Ramírez-Gil *et al*., 2014a; Ramírez-Gil and Morales, 2013).

According to the general symptoms associated with AWC, other causal agents reported in Colombia, are *Armillaria mellea*, *Cylindrocladium* sp*., Rosellinia* sp. *Fusarium* sp., *Rhizoctonia* sp*.,* and the nematodes *Helicotylenchus* sp*., Rotylenchulus* sp*.* and *Pratylenchus* sp. (Tamayo, 2007). The presence and possible associated symptoms of these pathogens have been reported, but without verifying their identity or their role in this pathology, given the lack of pathogenicity tests.

The relevance and incidence of each causal agent associated with AWC in Colombia depend on multiple factors, the genotype that interacted with soil, location (environmental and edaphic condition), stage of development of the host, and management of the production system. In each particular situation, *P. cinnamomi* Rands is considered the most important (Ramírez-Gil, 2018; Ramírez-Gil *et al*., 2017a; Rodríguez *et al*., 2017; Calle, 2017; Ramírez-Gil *et al*., 2014a; Yabrudy, 2012; Rodríguez *et al*., 2009; Tamayo, 2007).

Incidence, distribution and economic impact of avocado wilt complex

The incidence, distributions and economic impact of AWC in Colombia is practically unknown, given the lack of scientific reports at the national level of a systematic study that allows a broad inference in these topics, which have been addressed more at the regional level. Based on field observations, Tamayo (2007) reported that just during the crop's establishment, losses between 30 and 50% can be generated by this disease. However, he did not make reference to the causal agent responsible and the report it is not associated with a systematic evaluation. Meanwhile, Rodríguez *et al*. (2009) reported that for the Montes de Maria region in the Colombia Caribbean, the incidence of *P. cinnamomi* is greater than 40%. This information coincides with Yabrudy (2012), and Hernandez (2013) who declared that this problem caused the loss of a large quantity of the area cultivated with native avocado crops. Ramírez-Gil *et al*. (2014a) and Ramírez-Gil *et al*. (2017a), reported the incidence of AWC in Antioquia to be between 10% and 65%, where each of the agents involved in AWC presents different levels of importance and their distribution depending on multiple factors.

Analyses carried out in several regions of Colombia have shown that the incidence of AWC was largely determined by the technological level of the production system and the edaphoclimatic conditions. This evidence suggests that AWC increases its incidence in productive systems in which few agronomic, disease management practices are performed, and in seasons with extreme climatic conditions such as the high rainfalls that occur in the Niña phenomenon (Figure 3). In addition, Tamayo (2007) reports some departments in which different agent associated with AWC are present, but are needed to determine the dynamics of spatial-temporal, and causal agents in the different production systems that exist in Colombia (Figure 1).

From the work in Colombia, it can be affirmed that *P. cinnamomi* is the most important causal agent of AWC presents in all areas planted with avocadoes (Ramírez-Gil, 2018; Ramírez-Gil *et al*., 2017a; Rodríguez *et al*., 2017; Calle, 2017; Ramírez-Gil *et al*., 2014a; Yabrudy, 2012; Rodríguez *et al*., 2009; Tamayo, 2007).

In a recent study using ecological niche modeling (ENM) approach, the current and potential distribution of the five most important causal agents of the AWC in Antioquia was determined, confirming that *P. cinnamomi* has the largest range of distribution across Colombia (Ramírez-Gil, 2018; Ramírez-Gil, 2017b). In Figure 4 is shown a map of potential distribution of *P. cinnamomi*, generated from a modeling process using the ENM approach, based on reported presences for Hass avocado and all avocados (native breeds and commercial varieties). These results indicate that this pathogen is ubiquitous and its presence is determined by the host, and intensity by internal factors within each production system (edapholocimatic conditions, level of disturbance of

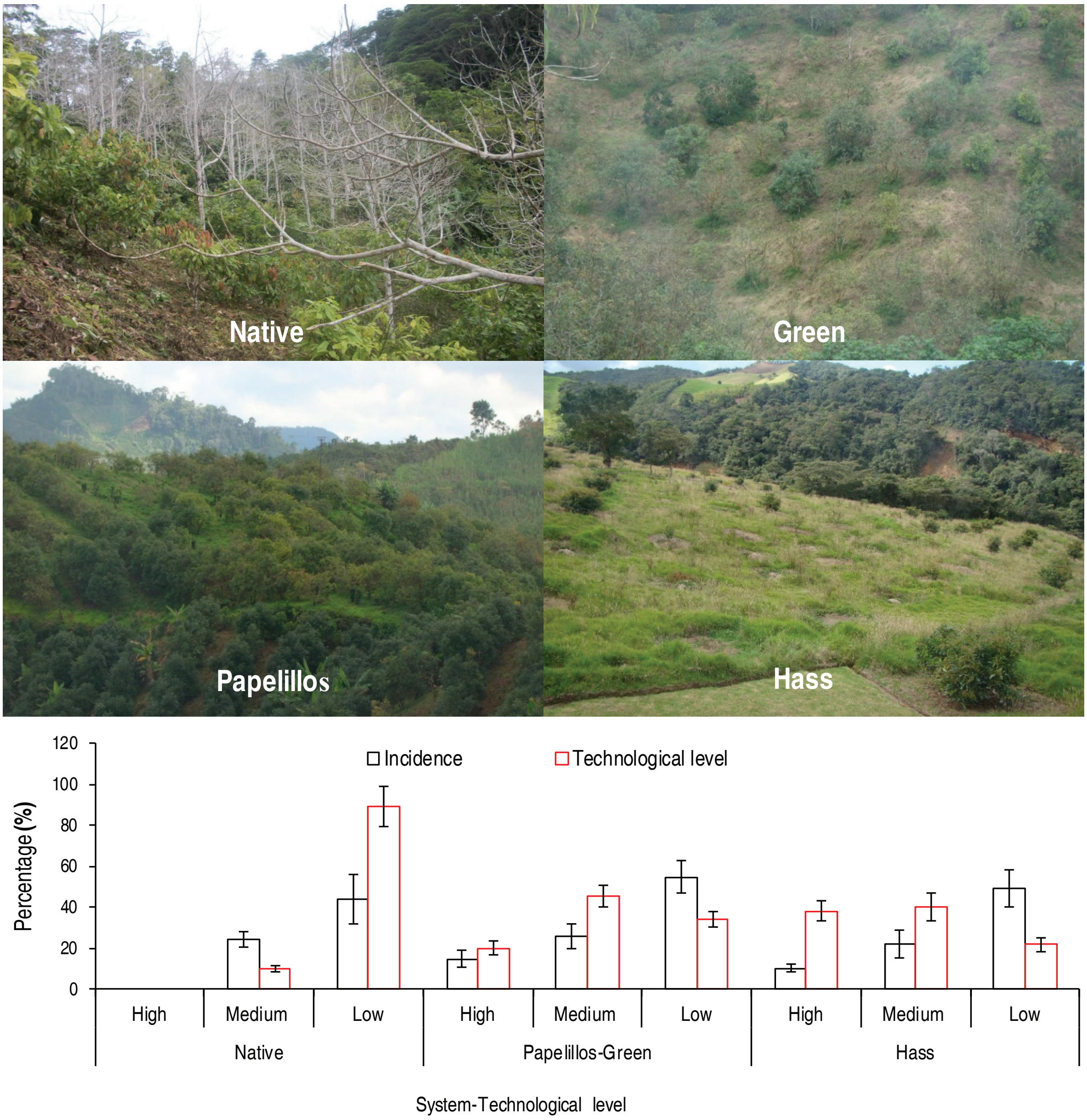

Figure 3. Overview of some avocado producing areas in Colombia and their relationship to the avocado wilt complex. Native avocadoes affected by avocado wilt complex in Turbo Antioquia. Green avocadoes affected by avocado wilt complex in Herveo Tolima. Papelillos avocadoes affected by avocado wilt complex in Aranzazu Caldas. Hass avocadoes affected by avocado wilt complex in La Ceja Antioquia. The bars represent the mean and confidence intervals, validated by Dunnett, Tukey and Kramer test. The overlap bars indicate that there are not significant differences between the periods evaluated (P>0.05). The data for the relationship between incidence and technological level of the productive system were collected in the field during the visit to different regions of Colombia following the methodology reported by Ramírez-Gil *et al.* (2017a). Native (Turbo in Antioquia department and Montes de Maria in Bolivar and Sucre department); Papelillos-Green (Herveo in Tolima department, Aranzazu in Caldas department, Montenegro in Quindio department, and Santa Barbara in Antioquia department); Hass (region of North, East and Southeast in Antioquia department).

the system, anthropogenic intervention, management practices, among others). The process of selecting the ENM was carried out in several steps. The first was associated with the selection of the calibration area (M), which is defined as an area accessible to the dispersion of the species, where its presence can be guaranteed (Barve *et al.*, 2011), for which 50 km buffer was used for the presence data of the native genotypes and commercial varieties. The following steps were associated with (i) reduction of the spatial autocorrelation of the presence data (Varela *et al.*, 2014). (ii) Selection of environmental variables based on NDVI obtained from MODIS images and topographic variables generated from a digital elevation model data at fine resolution (down to 250 m) (Ramírez-Gil *et al.*, 2018; Ramírez-Gil, 2017b). (iii) Reduction of the dimensionality of variables (Peterson, 2007; Peterson and Nakazawa, 2008; Varela *et al.*, 2014). (iv) Evaluation of the parameters of the algorithm (Warren and Seifert, 2011; Radosavljevic and Anderson, 2014). The models that presented best combinations of high information content, significance, and performance based on lower values of Akaike Information Criterion (AICc), partial receiver operating characteristic (partial ROC), and low omission of independent testing data records were selected (Ramírez-Gil *et al.*, 2018). The modelling process was developed using the maximum entropy algorithm (Maxent) (Phillips *et al.*, 2006). The statistical analysis and simulation were developed in the computational program R (R CoreTeam, 2017).

The magnitude of AWC in Colombia is evident in all of the production regions, where it has devastating effects and can destroy a great quantity of plants in short period of time, causing numerous losses which have yet to be quantified (Figure 3). In this sense and based on evaluations carried out in different regions of Colombia, it has been found that incidence of AWC was associated with different factors within the productive systems, especially those related to technological level (TL) (Figure 3). This concept (TL) is associated with a series of agronomic practices and management of the avocado system, which have been identified through statistical and mathematical relationships with different levels of incidence, severity and mortality of AWC (Ramírez-Gil *et al.*, 2017a; Ramírez-Gil *et al.*, 2014a).

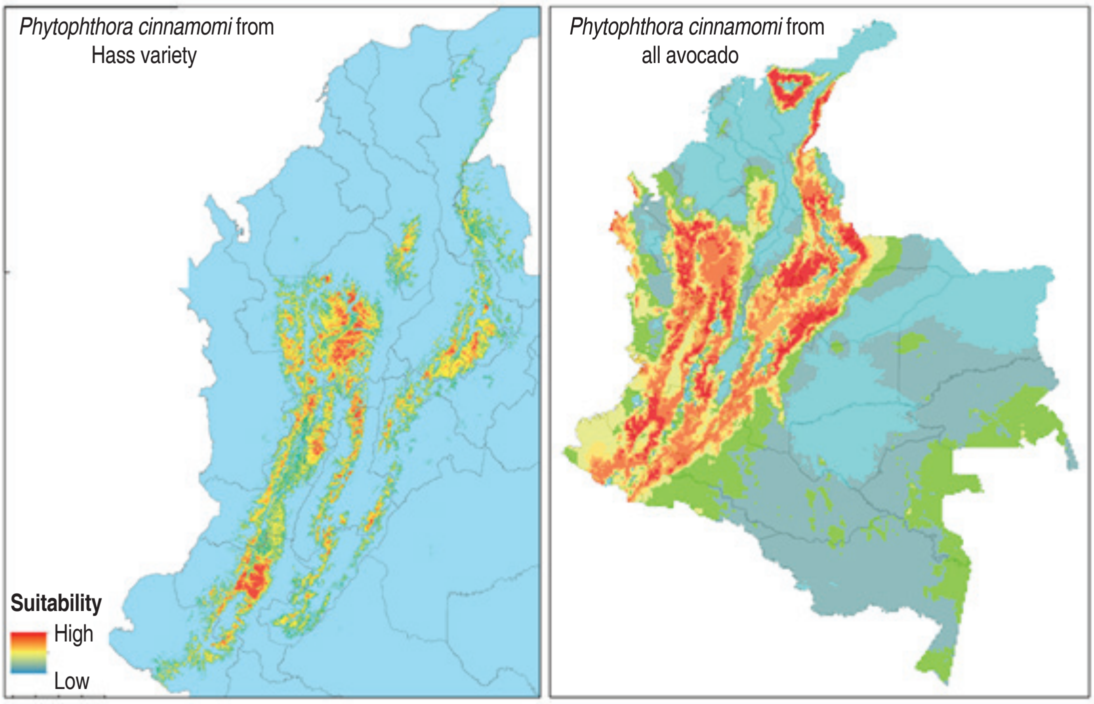

Figure 4. Current and potential distribution of *Phytophthora cinnamomi* from different native varieties of avocado in Colombia based on ecological niche modeling.

With regards to quantifying the economic impact of the AWC associated with *P. cinnamomi*, monitoring over the course of four years in some commercial plots of the Hass variety in Antioquia found that the productivity of trees infected that were not subjected to any management practice decreased by 89%, significantly impacting the top grade and high quality fruits. Moreover, this indicates that the failure to adopt a management strategy can generate a benefit/cost ratio lower than one, resulting in the loss of the initial investment (Ramírez-Gil *et al.*, 2017b). In a more detailed work in the three production regions of avocado in Antioquia (North, East and Southwest), economic losses due to AWC were 356, 340 and 325 USD in nurseries per one production cycle and 2340, 1702 and 2103 USD ha^{-1} associated with wilt destroyed plants and reduced in production and quality of fruits, with cost overrun of 225,372 and 287.9 USD ha^{-1} under field crops during seven years respectively (Ramírez-Gil *et al.*, 2017a).

Problems associated with avocado wilt complex

AWC has become a complex and difficult problem to manage, not only because of the economic losses it generates, but also due in large part to the fact that the symptoms caused by the different pathogenic agents, both biotic and abiotic, can easily be confused by plant diagnostics laboratories, technical assistants and farmers. This can generate incorrect diagnostics, which leads to inappropriate management practices, the majority aimed at *P. cinnamomi*. These practices can be effective if this is the causal agent responsible, but if not, they fail to resolve the problem, and the situation of the crop worsens, resulting in the decay and death of the trees in months or years of management (Figure 2 and 3). This situation was seen a plot in Altiplano Norte region of Antioquia, where seedlings were incorrectly diagnosed with *P. cinnamomi* and managed based on this diagnostic. This resulted in the death of all of the symptomatic plants. The correct etiological assessment found that the associated pathogen in that case was *C. destructans* (Ramírez-Gil and Morales, 2013).

The problem with AWC also leads to the excessive application of toxic products by the farmers. Guided by poor technical recommendations or their own initiative, they increase the dosage and frequency, and combine products, with the goal of mitigating the adverse impact of this disease. In statistical terms, and based on the data reported for the management of avocado diseases in Antioquia, Aproare Sat (2009) has noted that 68% of farmers use chemical control as the only management strategy, and that the most utilized products are phosphite and mancozeb + metalaxyl (Vásquez *et al.*, 2011). This implies immense economic costs for Colombia avocado farmers, since disease control forms part of the agricultural supplies rubric. Additionally, chemical control can have serious negative impacts on the environment and on human health, it may have implications for the safety of the product.

Principal factors predisposing to avocado wilt complex

The occurrence of AWC in the early stages of development is influenced by many factors, such as high precipitation, especially in extreme climatic phenomenon (La Niña), susceptibility in the initial stages in the field, planting of asymptomatic and symptomatic plants, and seedling with root atrophy (Figure 5) (Ramírez-Gil, 2018; Ramírez-Gil, 2013). Over time, it begins to show strong to medium spatial dependence and aggregation, and focal points are generated, that are associated with the lowest gradient zones in the terrain (Ramírez-Gil, 2013). With the goal of identifying other variables associated with these sources of infection, Ramírez-Gil (2013) analyzed the existing relationship between 32 edaphic properties and this disease, finding a strong relationship between a deficit in the surface movement of the water, retention of this element within the soil profile and the congruence of drainage networks (Figure 5). Together with the gradient of the terrain, this can influence other associated variables such as microbial fauna and some soil nutrients.

With respect to the variables associated with the management of the crop, Ramírez-Gil *et al.* (2014a) reported that the failure to implement set management practices leads to a greater occurrence of AWC (Figure 3). This indicates that abandoned crops or crops with technical problems (e,g., graft incompatibility, damage by insects, weed infestation, low soil fertility, disturbed soil, not pruning, among others) are more susceptible (Figure 5).

Management strategies of avocado wilt complex

Crop planning and cultural practices. The planning of avocado crops in Colombia is not based on technical factors, since the best edaphoclimatic conditions for sustainable development of the fruit are largely unknown. This leads to the establishment of the crop in terrains that are not apt for the plants and, to the contrary, can favor the presence of AWC, increasing its incidence and severity (Figure 3). This, in turn, indicates that some soil and climate factors are associated with AWC. Moreover, a series of management practices exists that can have a direct or indirect relationship with this disease, leading to a greater or lower susceptibility (Ramírez-Gil *et al.*, 2017a; Ramírez-Gil *et al.*, 2014a) (Figure 5).

Based on the experience developed for more than seven years in the commercial plantation of avocadoes, the fundamental parameters recommended is to avoid planting in clayey soils, with compacted layers, in flat areas with drainage problems, controlling the quality (genetic and phytosanitary) of nurseries plant, implementation of an integrated crop management, reduce excessive soil moisture by planting in monticules or installing drainage networks (Figure 5). Already in terms with the cultural practices in the plantations, is the basic integrated management associated with pests, diseases and weeds, performing balanced fertilization based on soil and leaf analysis, using mulching and organic material at zone with root influence, pruning for maintenance and phytosanitary reasons, and cleaning and waterproofing of the base of the stem (Figure 5).

Figure 5. Agronomic management practices associated with a higher or lower susceptibility of avocado wilt complex. A. Analysis of the soil profile to avoid compacted soil, clay soil or water table surface horizons; B. Avoid planting in low slope areas deficient in natural water drainage; C. Avoid planting in the consistency of water drainage networks; D and E. Reduce seedling with phytosanitary and genetic problems; F to H. Avoid using seedlings of poor quality (root atrophy, graft incompatibility, and reduce the infestation of weed; I to L. Implement management practices as use of mulching and organic material in the planting area, pruning for maintenance and phytosanitary reasons, waterproofing of the base of the stem, and planting in monticules or installing drainage networks and used an adequate management plat of pests, diseases and weeds, performing balanced fertilization based on soil and leaf analysis. The results are part of research in commercial avocado plots from the author.

Rapid and precise diagnosis
As was previously noted, AWC is caused by distinct causal agents. For this reason, the basis for its integrated management should be based on adequate diagnosis, which should include basic aspects of field symptomatology, cultivation in sterilized mediums, and macroscopic and microscopic observations of morphological characteristics. If necessary, this information should be confirmed using molecular characterization, additionally, must be careful in the determination of abiotic causal agents. A rapid and precise diagnostic of a pathological event is fundamental for managing the problem. Using this, effective control measures can be put in place, resources can be optimized and negative effects on the environment can be reduced. In addition, information can be generated regarding the interaction between the pathogen and the host.

In Colombia, the diagnosis of AWC has until now been based on observations of the symptoms that a particular causal agent can cause in its host. These observations are made by technical assistants and farmers. At a laboratory level, studies have been reported that have evaluated sensitivity to specialized diagnosis tests, such as the ELISA test for *P. cinnamomi* (Ciro *et al*., 2006), which isolates in culture mediums and morphological characterizations (Ramírez-Gil *et al*., 2014a); as well as the use of techniques based on the polymerase chain reaction, using general primers such as internal transcribed sequence (ITS), followed by sequencing of these regions (Ramírez-Gil, 2018; Ramírez *et al*., 2017a; Calle, 2017). Other techniques used the sequences of regions mitochondrial, and analysis with restriction enzymes (PCR-RFLP) (Calle, 2017; Leal *et al*., 2014).

In addition, in a high percentage of tissue infected with AWC, no microbial agent was isolated in traditional potato dextrose agar (PDA), leading to the use of specific media to colony growth, and with particular conditions for each group of microorganisms involved or the symptoms was associated with abiotic causal agent (Ramírez-Gil, 2018; Ramírez *et al*., 2014a). This result could indicate that the traditional technique used to isolation and identification of the causal agents AWC can failed. The AWC presents a challenge in the diagnosis, since they are causal agents associated with the root and stem system, so the simple visual inspection of symptomatology is not enough.

Use of resistant-tolerant rootstocks. Despite the importance of using resistant rootstocks as a management strategy, studies that have been performed in Colombia in this regard are low, making the commercial use of this option minimal. In regard to this strategy, one report characterized a series of genotypes, which were considered as escape since they did not show signs of being affected in areas with a high incidence of *P. cinnamomi* (Rodríguez *et al*., 2009). Based on germplasm collections, a potential material was found (NATU-001 from Tumaco Colombia) (Rodríguez *et al*., 2017). Actually, Profutales, a commercial nursery is using genotypes associated with Duke, but now no have result about of their behavior under Colombia conditions. These genotypes have been reported as tolerant to *P. cinnamomi* and are used commercially in many areas across the world (Zentmyer, 1980).

This indicates the need for a long-term program to be employed with the objective of characterizing the existent germplasms in Colombia and, based on this information, searching for materials with characteristics highly resistant to attacks by distinct causal agents responsible for AWC, which are also able to adapt to the country's various agricultural regions. In addition, Ramírez-Gil (2018) reported that the native genotypes of *P. americana* is grouped by race, giving rise to different types of behavior according to the causal agent infected. This situation makes it necessary to use multiple criteria in the selection processes of materials for commercial use.

Meanwhile, the growth of the area planted with avocado in Colombia has resulted in the need for continuous production of seedlings, which should comply with two basic parameters: phytosanitary quality and genetic identity. Currently, there is a constant failure to comply with these two criteria (Figure 5). This is a limiting factor that strongly influences the dispersion of pathogens, losses associated with AWC and the competitiveness of this economic activity.

Physical practices. One of the physical practices used for the management of soil pathogens is the technique of solarization, which achieves its effect by increasing temperature, thus eliminating certain infective propagules. In Colombia, this technique is not commonly used in commercial crops, despite the fact that under experimental conditions its effects on the management of *P. cinnamomi* have been promising, but presented several limitations, especially the need to achieve high temperatures for good effectiveness, since under experimental conditions the temperature has not exceeded 35 °C in a plot when this technique was been evaluated by the author, but in order to inactivate pathogenic structures of *P. cinnamomi* and *Verticillium* sp., pathogens involved in AWC, it is necessary higher temperatures than 35 °C (Gallo *et al*., 2007; Pullman *et al*., 1981) (Figure 6A). The infrequent adoption of this technique is due to the fact that the farmers see it as a cumbersome and costly alternative, although in some regions it is used in an indirect manner, since plastic coverings are used with the objective of decreasing moisture in the soil and/or eliminating weeds.

A more generalized practice is the use of extreme pruning, a strategy that the author recommended used when the plants present an advanced state of the disease and other alternatives have failed, at which point other management measures are economically unviable (Figure 6B and 6C). This strategy produces a rapid response under this condition (advanced state of the disease), as a direct consequence of the compensation of the root system for the aerial part (Sanclemente *et al*., 2014), which induces the production of new roots and foliar buds (Figure 6A). At the moment this occurs, the application of other management practices should be initiated (Ramírez *et al*., 2017b), since if this is not done, the pruning by itself is not sufficient to control *P. cinnamomi* (Figure 6B). Under field conditions, this strategy has proven to be useful, when disease status is greater than 3, for which the use of a specific scale is recommended for each causal group associated with the AWC (Ramírez-Gil, 2018).

Figure 6. Physical, microbiological and chemical management strategies in avocado wilt complex. A. Solarization; B. Plant with *P. cinnamomi* in stage 4 of disease development scale; C. Severe pruning for management of *P. cinnamomi*; D. Cankers in the base of the stem caused by *P. citrícola*; E, F. Cankers surgery and application of a waterproof, curative and preventive pasta; G, H. Addition organic material and mulching in the planting area; I, J. Application of a fungicides with phosphite how active ingredient into the stem using injectors and clinical syringes; K, L. Application of a fungicides with metalaxil how active ingredient in drench and powder in the cankers the stem. The phots are representation of many practices associated with AWC management under assays in field developed by the author.

Another mechanical or physical strategy for the management of AWC that the author recommended is the removal of the source of inoculation, which can be done for those plants that present cankers at the base of the stem caused by *P. cinnamomi*, *P. palmivora*, *P. citricola* and *P. heveae* (Figure 5D) or stem rot caused for *Verticillium* sp. and *L. theobromae* (Figure 2). The correct use of this practice consists in the removal of the entire affected area in order to guarantee that the remaining tissue is healthy, followed by the application of a paste made up of a contact active ingredient (mancozeb, 1 g L^{-1} a.i), a systemic active ingredient (metalaxil 0.5 g L^{-1} a.i) and an impermeable paint (Figure 6E and F). This management practice has produced satisfactory results when it is done correctly and the appropriate maintenance of the plant is ensured (Figure 7C).

Microbial alternatives, mulching and organic material. In Colombia, various studies have been performed with the objective of evaluating the effects of distinct microorganisms on reducing *P. cinnamomi,* identifying important metabolites with potential use in commercial products (Ramírez *et al*., 2015; Granada *et al*., 2018), but application in yields has been reduced. In this regard, Ramírez-Gil *et al*. (2014b) evaluated, under greenhouse conditions, the interaction of *Glomus fasciculatum*, *Trichoderma harzianum*, *Pseudomona* sp. and *P. cinnamomi*. They found that the greatest reduction of the disease occurred with the use of *T. harzianun*, although they also reported a possible antagonism between *G. fasciculatum* and *T. harzianun,* indicating the highly complex nature of soil microorganisms and their use in commercial agriculture. In another work Leal *et al*. (2014) found action of a strain of *T. harzianum*, through colonization on *P. cinnamomi*.

The limits of these studies rest in the fact that their evaluation and results occur in controlled conditions and not in the field, meaning they are only potential alternatives for the management of a particular microorganism. The concept of microbiological regulation operates through an ecological equilibrium, a condition which prevents the population of

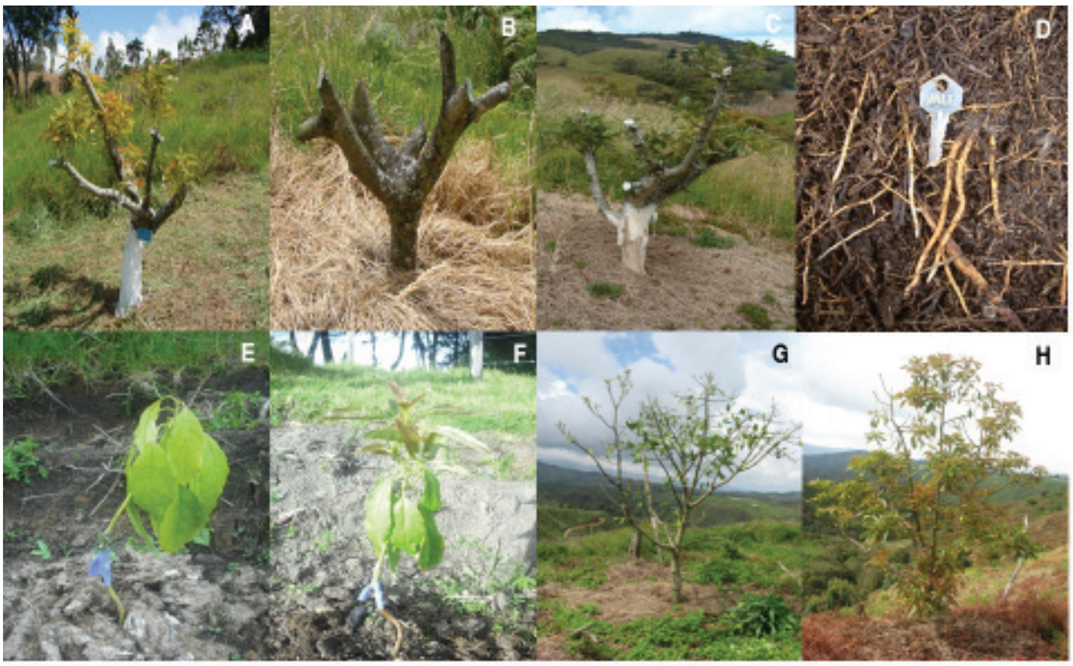

Figure 7. Results of management practices in avocado wilt complex. A. New leaf buds after performing severe pruning; B. Dead plant after performing severe pruning; C. Recovery of plant infected with *P. citricola* after performing surgery and complementary treatment; D. Induction of viable roots in plants with added mulching; E, F. Seedling infected with *P. cinnamomi*, and recovery after implementation of integrated management strategy; G, H. Adult infected with *P. cinnamomi*, and recovery after implementation of integrated management strategy. The phots are representation of results associated with AWC management under assays in field developed by the author.

a certain pathogen from expressing its potential, thus the symptoms of the disease remain minimal. This situation can be generated through the addition of extra organic material, mineral amendments, cow manure and mulching evaluated by the author with excellent results (Figure 6G and 6H). The use of these alternatives has a revitalizing effect on microbial soil fauna, leading to the proliferation of beneficial microorganisms such as fluorescent *Pseudomonas* spp., aerobic endospore-forming bacteria (BAFE), cellulolytics, *Trichoderma* spp., and enzymatic activity of cellulose. This in turn leads to a decrease in the severity of the disease caused by *P. cinnamomi* (Ramírez-Gil *et al*., 2017b; Leal *et al*., 2014; Ramírez-Gil *et al*., 2013). In addition to improving the nutrition of the plant and producing a higher quantity of viable roots (Figure 6C).

Use of fungicides. Given the lack of knowledge of the fact that AWC can be caused by distinct causal agents and even have abiotic origins, the use of fungicides in the cultivated plots is restricted to those recommended for *P. cinnamomi*. Of these, phosphite fungicides is notable, and in particular potassium phosphite, which is applied both to the leaves and to the soil as a drench, though the most common manner of application has been to inject it into the stem using injectors (Figure 6I) and clinical syringes (Figure 6J). Another active ingredient that is commonly used is metalaxyl, which is applied as a drench to the soil (Figure 6K) or in powder form to lesions created or to cankers in the base of the stem of affected trees (Figure 6E and 6M). The use of phosphite and metalaxyl have presented good results in greenhouse and field conditions in the management of *P. cinnamomi* (Ramírez-Gil *et al*., 2017b; Leal *et al*., 2014; Ramírez-Gil *et al*., 2014b; Tamayo, 2007).

The use of chemical compounds has been widespread in Colombia, since they produce rapid effects and lead to the partial recovery of avocado plants affected by *P. cinnamomi*. However, it is important to keep in mind that, when used alone as a management practice, this does not lead to the full recovery of the plant, and after a certain period of time the symptoms return and the progress of the disease continues (Ramírez-Gil *et al*., 2017b). Based on experience of the author the appropriate time to apply chemical compounds is during the early stages of the development of the disease. One problem associated with the excessive use of phosphites and metalaxyl is that these active ingredients are specific for oomycetes, for which reason the effect is reduced in microorganisms belonging to other groups, such as fungi. Additionally, in a field setting the diseases in the plants can often have an abiotic origin. It has also been reported that the fungicide with the active ingredients mancozeb + metalaxyl decreases the native populations of fungi in the soil (Ramírez-Gil *et al*., 2013). Another problem is that the continuous use of these chemical compounds for the management of *P. cinnamomi* can exert a strong selective pressure on the populations of these microorganisms, in some case generating resistance or decreasing sensitivity to the metalaxyl (Darvas and Becker, 1984) and even the phosphites (Dobrowolsk *et al*., 2008).

It is important to report that the inappropriate use of phosphites can cause plant phytotocity, which is often observed under field conditions by the author, given the excessive application and the use of high doses of this active ingredient in commercial avocado crops.

Integrated management plan. The experiences until now in the management of *P. cinnamomi* in Colombia have been based on experimental data (published, and unpublished by the author), and the rest of the world demonstrate that the use of individual management strategies does not decrease the losses that this disease can cause (Ramírez-Gil *et al*., 2017b; Tamayo, 2007; Zenmeyer, 1980). They can reduce the adverse effects, but the recovery of the plant and its productivity requires the combination of a series of strategies, all combined into one integrated management plan (Ramírez-Gil *et al*., 2017b; Zenmeyer, 1980). Because of this, if the goal is to attain a sustainable farming system, the correct choice is to perform the greatest quantity of practices that might allow for a healthy coexistence with *P. cinnamomi* and the other pathogens that cause wilt complex. A clear example has been the successful management in a field setting of *P. cinnamomi* in both small plants (Figure 7E, 7F) and adult plants (Figure 6G and 6H), through the application of metalaxyl as a drench, the application of potassium phosphite to the leaves or via injection, additions of organic material such as cow manure, mulching, mineral additions rich in calcium, phosphorous and silicon. The results of these management practices have proven to be successful, which have been replicated experimentally in several commercial farms in the Antioquia department.

In addition, there are few scientific bases for the management of avocado root rot caused by causal agents different to *P. cinnamomi*, therefore accurate and prompt diagnosis are not routinely performed and integrated management strategies are not readily available. Besides, little adoption of technology by producers is observed, mainly because they represent high costs or are difficult to apply under field conditions. To increase technology adoption in the avocado industry, new methods should be inexpensive and user-friendly.

Use of technology of information. From many studies (Ramírez-Gil *et al*., 2017a; Ramírez-Gil *et al*., 2017b; Ramírez-Gil *et al*., 2014a; Ramírez-Gil, 2013), an early warning system to support an integrated management system was developed. This tool included three aspects, prediction, diagnosis and recommendation. The first was based on mathematical relationship between AWC and environmental variables. The second was developed in four strategies (i) image recognition, using a machine learning algorithm, (ii) decoding of the image in the RGB, (iii) reflectance analysis, and (iv) diagnosis kit. These tools were integrated by means of a neural network to produce a response, which was used to determine the causal agent associated with the AWC. The third was associated with generating a recommendation for the integrated management of a causal agent of AWC. This platform was developed in a web page, and an app for phone with android system (Ramírez-Gil, 2018; Ramírez-Gil, 2017b).

Prospects for the future

The biggest challenge for the future of avocado cultivation in Colombia and all the world will be finding ways to adapt production to climate change, since it is believed that future climate scenarios in Colombia will entail the ever more frequent occurrence of the ENSO phenomena (La Niña and El Niño) (Ochoa and Poveda, 2004). Under conditions of high climatic variability (El Niño and La Niña) it has been found that the AWC can present significant changes in variables such as incidence, severity and mortality, in special under high precipitation (La Niña), where this pathology increases strongly (Ramírez-Gil, 2018). In a preliminary study, Ramírez-Gil (2018) developed two mathematical models, whose aim were to predict incidence and severity of wilt complex batch cultivated. From these we found that this developed based on precipitation and this interaction in the soil profile showed a high prediction capacity in these two variables, indicating high correlation with the disease.

This is a worrying scenario, given that soil moisture is one of the most serious limiting factors for this crop, and one that can trigger wilt complex. These studies should be complemented with the designing of rapid diagnostic tests of all the causal agents responsible for this disease. Using these, a research program should be put in place that will allow for the discovery of integral solutions to this problem that can mitigate its adverse effects through economically viable management practices, which are available to producers and easy for them to adopt. This is how Ramírez-Gil (2018) evaluated different strategies to mitigate the adverse effects of climate variability in *P. americana*, finding that the mulch is an excellent strategy, besides the promising report that within this species well the many native materials which have a different behavior to the different moisture contents in the soil.

It is also necessary to study and better understand soil microbiota, which could become a fundamental tool for confronting adverse situations. In preliminary studies it has been reported that the inoculation of beneficial microorganism in seedlings can increase root production, a mechanism that can improve plant adaptation to adverse conditions such as sudden climatic changes and attacks by soil pathogens (Ramirez-Gil *et al*., 2014b). Additionally, it is believed that excess moisture in soil provokes biochemical changes in plants, amongst them an increase in the production of ethylene, resulting in progressive decay and eventual death (Sanclemente *et al*., 2014; Stolzy *et al*., 1967). This phenomena can be reversed with microorganisms, including plant growth promoting rhizobacteria (PGPR), which can suppress the route of the ethylene, thanks to the inhibition of aminocyclopropane-1-carboxylate (ACC), one of the precursor enzymes for this phytohormone (Arshad *et al*., 2008; Glick *et al*., 2007). About this topic Ramírez-Gil (2018) found that moisture in the soil, have a close relationship with changes in precipitation resulting from climate variability, and that the amount of water in the soil profile, has a strong influence on soil microbial populations, suggesting a strong importance for soil drainage capacity in AWC.

It is also important to announce the need to carry out advanced studies in the host-pathogen interaction at cellular and molecular level, in order to identify tolerant genotypes in Colombia and to understand the pathogenic process with the aim of designing best management practices of AWC.

CONCLUSIONS AND RECOMMENDATIONS

The technological challenges presented by avocado cultivation in Colombia prevent the production systems from being competitive. For this reason, it is necessary to work to solve these problems in order to be able to take advantage of free trade agreements, in which the fruit sector, and particularly avocado crops, has very good prospects. If wilt complex is not dealt with in a technical manner, in the future this could have negative consequences for the potential exportation of this fruit to foreign markets that have a demand for a healthy and innocuous food, which complies with the basic criteria of good agricultural practices (GAP).

One important factor that has made diseases like wilt complex and other problems a major limiting factor in avocado production in Colombia is the fact that its management has been based on the use of foreign technologies, which in many cases are not applicable in Colombia. This situation points to the need for the country to instate a highly scientific and innovative research program that produces original technologies, without economic limitations, that are operative and adoptable, and will lead to a decrease in negative impacts on the environment, protect human wellbeing and health and, furthermore, have economic benefits. A technical proposal generated through a full research process, in addition to proper coordination between the public and the private sectors, would lead to an economically sustainable system, which could have a revitalizing effect on the country by strengthening social, environmental and cultural dimensions.

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