Essay

Current situation of postharvest handling and fungal diseases of avocado 'Hass' for export in Michoacán, Mexico

Juan Antonio Herrera-González[1, 2]
Silvia Bautista-Baños[3]
Samuel Salazar-García[4]
Porfirio Gutiérrez-Martínez[1§]

[1]Tepic Technological Institute-Comprehensive Food Research Laboratory. av. 2595 Technology, Lagos of the Country, Tepic, Nayarit, Mexico. CP. 63175. (juanherreragonzalez@gmail.com). [2]Uruapan Experimental Field-INIFAP. Av. Latinoamericana 1101, Col. Revolución, Uruapan, Michoacán, Mexico. CP. 60150. [3]National Polytechnic Institute-Center for the Development of Biotic Products. Yautepec-Jojutla highway km 6, CEPROBI 8, San Isidro Yautepec, Morelos, Mexico. CP. 62730. (sbautis@ipn.mx). [4]Santiago Ixcuintla Experimental Field-INIFAP. Junction of the international highway México-Nogales km 6, Santiago Ixcuintla, Nayarit, Mexico. CP. 63300. (salazar.avocado@gmail.com).

[§]Corresponding author: pgutierrez@ittepic.edu.mx.

Abstract

Mexico is the world's leading producer and exporter of avocado 'Hass'. Due to its nutritional characteristics in human health, the avocado 'Hass' has gained great popularity around the world. The objective of this review was to know the current state of avocado harvest and post-harvest management, as well as to review the infection process of the fungi *Colletotrichum* spp. and *Lasiodiplodia theobromae*, which cause the diseases called anthracnose and pedicle rot, respectively. These pathologies manifest themselves during postharvest maturation and storage. Currently, the main means of control of these microorganisms is a complex of the fungicides azoxystrobin + fludioxonil, the application of which is allowed only for exports to the United States of America. In Mexico, only the state of Michoacán can export avocado to the United States of America, but it seeks to increase exports to other international markets and integrate other producing regions, so it is necessary to look for alternatives to control postharvest diseases. Through the knowledge of the intrinsic protection mechanisms of the fruit, the bases could be laid to develop and evaluate new control models.

Keywords: *Colletotrichum* spp., *Lasiodiplodia theobromae*, chemical control.

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Mexico is the world's largest producer of avocado (*Persea americana* Miller) with a volume close to 2.2 million tons. The largest producer is the state of Michoacán with 77% of the total, followed by Jalisco (9%), States of Mexico (4%), Nayarit (3%) and Morelos (2%) (SIAP, 2018b). The main cultivar is Hass and its demand has increased in recent years because it is a food of high nutritional and functional value; in addition, in the countries where it is produced it has a great economic impact (Araújo *et al*., 2018). The main export destination for Mexican avocados is the United States of America, although exports to Canada, Asia, Europe and Central America are also increasing.

Avocado is a climacteric fruit (accelerated increase in autocatalytic ethylene biosynthesis after harvest, which coincides with an increase in fruit respiration), which makes it difficult to preserve and sell it to distant markets, reducing its quality of consumption, life shelf life and its commercial value (Ramírez-Gil *et al*., 2020). One of the main causes of postharvest losses is due to diseases such as anthracnose and pedicle rot, caused by the fungi *Colletotrichum gloeosporioides* and *Lasiodiplodia theobroame*, respectively.

Usually the control of these microorganisms is done by applying synthetic fungicides both pre and postharvest, which can cause resistance in pathogens, as well as damage to health and the environment.

In addition to this, there are markets with low tolerance in the maximum allowed limits of chemical residues in the fruit. In this work, the current state of harvest and post-harvest handling of avocado 'Hass' is disclosed, with emphasis on the state of Michoacán, as well as reviewing the infection process of the main pathogenic fungi that affect its export.

Avocado industry in Mexico

Due to the different environmental conditions of the avocado producing areas in Mexico, it presents wide genetic variability and great adaptability to the climate and soil. After the arrival of the Spanish to Mexico, the avocado spread to different parts of the world where it is currently cultivated because the tree adapts to a large number of environments (Barrientos *et al*., 2008).

Avocado exports to the United States of America (86% of Mexican exports) are carried out through the Association of Producers and Packers Exporters of Avocado of Mexico (APEAM), which is in charge of facilitating the necessary work for the export and reconcile compliance with the phytosanitary, safety and certification standards of the Ministry of Agriculture and Rural Development (SADER) and the United States Department of Agriculture (USDA) that ensure the production of healthy and high quality fruit.

APEAM has more than 25 thousand partner-producers and 54 affiliated export packing companies, in addition, it sponsors promotional campaigns (Avocados from Mexico, https://avocadosfrommexico.com/), as well as research and technological development, among others.

Although the export of avocado is contemplated in the Mexico-United States of America-Canada trade agreement (T-MEC), the state of Michoacán is the only one that can export fruit to the United States of America and only those municipalities free of the following quarantine pests: large bone borer (*Heilipus lauri*), branch borer (*Copturus aguacatae*), small bone borer (*Conotrachelus aguacatae* and *C. persea*) and avocado bone borer moth (*Stenoma catenifer*) (NOM-066-FITO -2002, 2005). The high volume of avocado exported from Mexico to the United States of America is due to the fact that the transfer time is between 48 and 72 hours and is carried out in tractors. However, the rest of the producing states (Jalisco, State of Mexico and Nayarit) that cannot export to the United States of America, allocate their fruit to the domestic market and exports to more distant markets (Canada, Japan, Asia, Europe, as well as such as Central and South America) that in most cases its transport is maritime and with transfer times of between 28 and 40 days (SIAP, 2018ab).

The majority of Mexican avocado exports are destined for the US market, so it is important to continue strengthening our presence in this market; however, it is necessary to do the same with other markets to diversify the economic risk of concentrating on a single market.

Nutritional value of avocado

The avocado fruit is considered a 'functional' food, since it prevents the development of chronic degenerative diseases. Contains all essential amino acids and in greater quantity than other fruits (1-3% protein content), as well as a mixture of soluble (2.1%) and insoluble (2.7%) fiber ideal for diets (Cowan and Wolstenholme, 2016). The pulp contains between 70% and 78% of water and 9-15% of oil at the time of harvest and at maturity of consumption between 77% and 67% of water and 10-19% of oil (Herrera-González *et al*., 2013a) and for each gram of water lost, the oil content increases by 1 g (Cowan and Wolstenholme, 2016).

Avocado pulp is also rich in fatty acids and they are composed of: 1) neutral lipids (tri, di and mono-acyl-glycerols); 2) phospholipids; and 3) glycolipids and free fatty acids; also, it is rich in mono and polyunsaturated fatty acids (oleic-C18:1, linoleic-C18:2 and palmitoleic-C16:1) and does not contain cholesterol.

Therefore, the consumption of these fatty acids can reduce cardiovascular diseases, reducing the levels of low-density lipoproteins in the blood and increasing the levels of high-density lipoproteins (Krumreich *et al*., 2018). Among the vitamins and phytochemicals that have been reported in avocado are vitamins C (ascorbic acid), A, B-6, E (alpha tocopherol) and K (phylloquinone); in addition, it contains thiamine, riboflavin, niacin, pantothenic acid, folic acid, choline, betaine, beta carotene, alpha carotene, beta cryptoxanthin, lutein, zeaxanthin, beta tocopherol, gamma tocopherol and delta tocopherol (US Department of Agriculture, Agricultural Research Service, 2019). Also, the avocado pulp is rich in minerals such as P, K, Ca, Mg, Na, Fe and B (Bautista-Baños *et al*., 2019).

During avocado ripening, the content of sugars such as glucose and fructose increases, in addition, certain unusual seven-carbon sugars are present (D-mannoheptulose and its reduced form perseitol) (Hurtado-Fernández *et al*., 2018). Therefore, the consumption of avocado helps with the loss of body weight, accelerating the metabolism, reducing the levels of low-density lipoprotein cholesterol and maintaining the levels of high-density lipoprotein. It has chemopreventive action on carcinogenic inflammation (prostate cancer), arthritis and heart disease and is associated with a reduction in the risk of metabolic syndrome (high blood pressure, high blood sugar and excess body around the waist) (Dreher and Davenport, 2013).

Harvesting and post-harvest handling of avocado for export

The Mexican standard NMX-FF-016-SCFI-2006 establishes the minimum requirements that the avocado ‘Hass’ fruit must meet to be harvested. For example, the minimum dry matter content in the pulp must be 21.5%, whole, visibly healthy, free of insects, without damage by pests and without strange smells and tastes. Although the standard only contemplates cv. Hass, other cultivars such as ‘Méndez Num. 1’, are harvested and stored the same as ‘Hass’; however, the optimal harvest maturity of ‘Méndez’ is reached with higher dry matter (22.7% dry matter) than that of ‘Hass’ (Herrera-González *et al*., 2017b).

The determination of dry matter according to the standard is carried out by drying in a microwave oven to a constant weight of the sample, but it is a destructive method. It can also be performed using near infrared spectroscopy, which by reflactance determines the dry matter content of the fruit without destroying it (Osuna-García *et al*., 2018).

The fruits are harvested with scissors or hooks with knives mounted on a pole and placed in collection bags that, once full, are emptied into 28-30 kg plastic boxes. Subsequently, the boxes are transported to the packinghouse in sealed trailers or trucks (Figure 1). Once there, the fruit is unloaded in the collection area where it rests for 12 hours so that the temperature of the fruit decreases (Herrera-González *et al*., 2017c). The selection and packing begins with the emptying of the fruits in the bands and rollers that take them to the brushing, followed by a manual selection where fruits with visible damage are discarded.

Figure 1. A) avocado fruits on the tree; B) harvest with hook and scissors; C) cutting the peduncle (pedicel) and placing it in a bag; D) carry boxes of 28-30 kg; E) trailer transfer from the harvest site to the truck; and F) stowage of boxes and transport to the packing house (Herrera-González *et al*., 2017c).

The healthy fruits continue their route to the labeller and the sorter by size, which drops them into the packaging hoppers, where the person performs a quick inspection of each fruit and if it meets the quality they are placed in cardboard boxes (Figure 2). When the batch is complete, it is transferred to a pre-chilling room (8-10 °C) for 12 h (to avoid thermal shock) before moving to the storage chambers (4-5 °C), where they will wait between 12 and 48 hours for the boarding to be completed (NMX-FF-016-SCFI-2016, 2016; Osuna-García *et al*., 2017; Herrera-González and Salazar-García, 2018).

Figure 2. Stages of the avocado ‘Hass’ selection and packing process for export: A) emptying of fruits; B) brushing; C) manual selection; D and E) automatic; F) labeling; G) fall into the hopper; and H) packed (Herrera-González and Salazar-García, 2018).

Technology during storage

Refrigeration is the most widely used preservation technology in avocado. The optimum temperature for ‘Hass’ is 6 °C with a relative humidity of 90-95%; however, the temperature can be lowered to 2 °C when the fruit has a high content of dry matter (>30%) and oil (end of the production season, >15% where the incidence of cold damage is lower) (Osuna-García *et al*., 2017). For the most distant destinations (Europe and Asia), refrigeration is complemented with the use of controlled atmospheres, in which the concentration of O_2 is reduced (2-5%) and that of CO_2 is increased (3-10%). These concentrations must be regulated and maintained during transport. Controlled atmospheres make it possible to decrease respiration rate, autocatalytic ethylene production, enzymatic activity, and maintain firmness (Prabath-Pathirana *et al*., 2011; Bill *et al*., 2014).

Another complementary alternative to refrigeration and more commonly used in Mexico are ethylene absorbents, such as potassium permanganate ($KMnO_4$). Because avocado is a climacteric fruit, ethylene production increases during storage, so each box in the batch (700-1 100 boxes of 11 kg) includes a small bag of $KMnO_4$, which is relatively inexpensive (> USD $0.25 per bag) (Kant, 2018; Perato *et al*., 2018). However, both refrigeration and controlled atmospheres reduce the physiological processes of the fruit, but not the presence of pathogens (Figure 3).

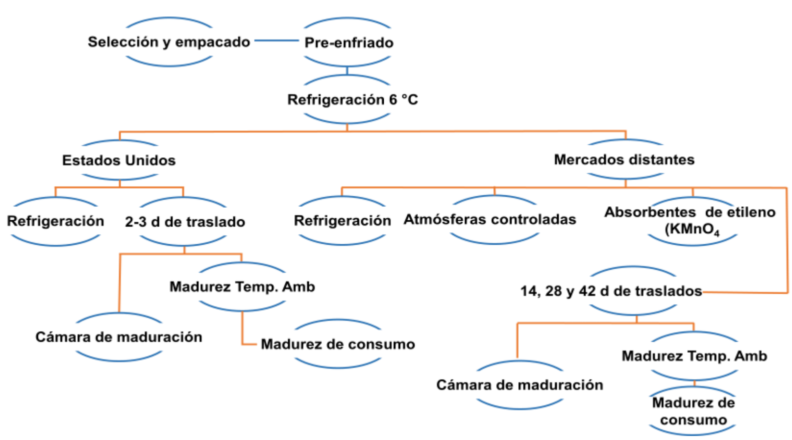

Figure 3. Process followed by avocado ‘Hass’ fruits after packing and storage.

Fruit development and ripening

The avocado fruit is a drupe, with sclerified tissue in the seed and its pericarp is composed of endocarp, exocarp and mesocarp (Figure 4a) (Barrientos *et al*., 2008). The development of the fruit begins in the anthesis, although for its monitoring in the field it has been divided into several stages of development, E-I: the fruit has a diameter of 35-45 mm; E-II: the predominant activity is cell division and defense mechanisms of the fruit develop, which has a diameter of 50-60 mm; E-III: the size of the fruit is increased (diameter 60-70 mm) due to cell elongation, in addition, in this phase a large amount of antioxidants is produced in the exocarp and to a lesser degree, in the mesocarp, where oil also accumulates; finally, physiological maturity is reached (> 70 mm in diameter, 21.5% dry matter for 'Hass') (Tesfay *et al*., 2010; Medina-Carrillo *et al*., 2017) (Figure 4b).

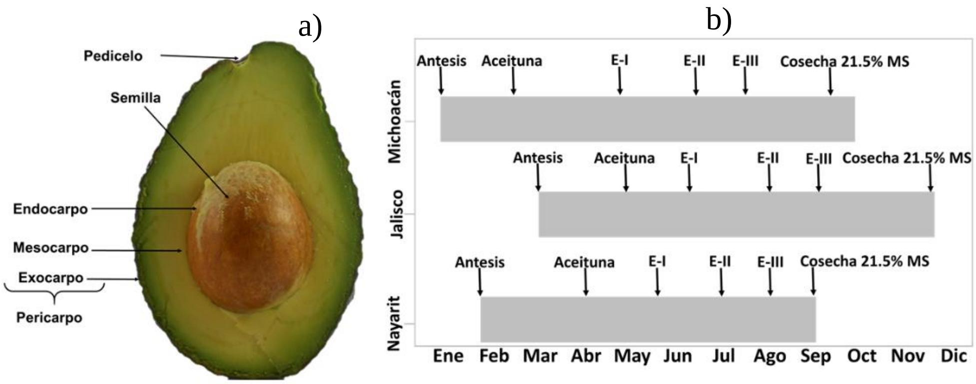

Figure 4. a) Parts of the avocado 'Hass' fruit; and b) development stages (olive, I, II, III and harvest) of the fruit in three producing regions of the Pacific of Mexico (adapted from Medina-Carrillo *et al*., 2017).

To harvest the avocado fruit in Mexico, between 7 and 10 months are required from anthesis to physiological maturity, although the beginning of this phase may vary depending on the climate, altitude, soil moisture condition, pre-harvest management of the production area of avocado (Salazar-Garcia *et al*., 2018), market demand and the size (caliber) of fruit required.

The postharvest maturity of the 'Hass' fruit is a complex process of physiological, biochemical, genetic and enzymatic changes that lead the fruit from physiological maturity to senescence. These metabolic processes are responsible for the characteristics and quality attributes of the fruit. During ripening, the change in the texture and firmness of the pulp are the consequence of changes in the metabolism of cell walls by the enzymes cellulase, polygalacturonase, pectinesterase and β-galactosidase, as well as the accumulation of sugars, reduction of acids organic substances, degradation of skin pigments to turn black (accumulation of anthocyanins), production of volatile compounds and aroma, in addition to the increase in respiration and ethylene production (Figure 5) (Handa *et al*., 2012; Cowan and Wolstenholme, 2016; Hao *et al*., 2017; Pedreschi *et al*., 2019).

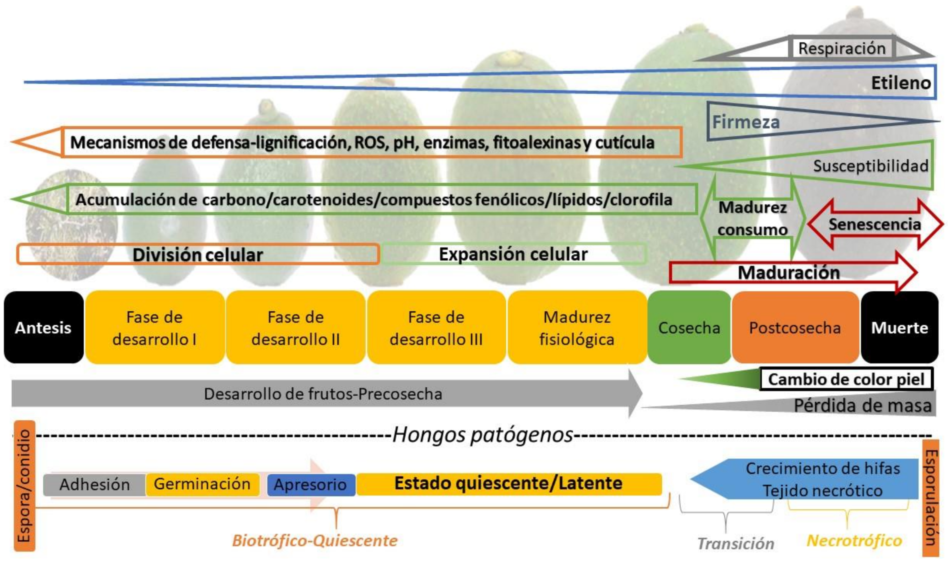

Figure 5. Processes in the development of the avocado 'Hass' fruit from anthesis to postharvest senescence and the process of infection and development of pathogenic fungi in the fruit.

Post-harvest losses

Postharvest deterioration of avocado is mainly caused by the incidence of fungal diseases. Among the most important are anthracnose and pedicle rot, the symptoms of which appear mainly during the ripening process and are enhanced due to mechanical damage, physiological disorders, inadequate storage temperature, long periods of refrigeration, harvest time and fruit age, among others (Arpaia *et al.*, 2018; Bowen *et al.*, 20188). These diseases are the main source of losses and occur in most avocado producing areas of the world (Silva-Rojas and Avila-Quezada, 2011).

In an experiment carried out by Herrera-González *et al*. (2017d) the application of fungicides days before harvest reduced the incidence of anthracnose and pedicle rot to less than 10%, compared to the control (without fungicide) that presented up to 60% incidence of these diseases.

The loss due to fruit rot has a strong economic impact, since the price per kilogram of conventional avocado for the United States of America ranges between 24 and 42 pesos (USD $1 to 2.50), for Japan between 26 and 38 pesos (USD $1 to 2) and organic for any market between 37 and 42 pesos (USD $1.50 to 2.50). Therefore, the rejection of fruit is important when it loses its aesthetic characteristics and quality attributes, detracting from commercial prices. The previous loss is added to those that occur during harvesting and packing, which is close to 6% per ton of harvested fruit (Berry *et al.*, 2017; Bustos and Moors, 2018; Ramírez-Gil *et al.*, 2020).

Pathogenic fungi of the fruit during post-harvest handling

It has been mentioned that the main diseases of the avocado fruit are anthracnose and pedicle rot, which can be caused by various microorganisms. In general, the main pathogens are *C. gloeosporioides* and *L. theobromae* (Twizeyimana *et al*., 2013; Galsurker *et al*., 2018).

In Mexico, *C. gloeosporioides*, *C. acutatum*, *C. boninense*, *C. godetiae*, *C. karstii* and *C. fructicola* have been found as the species causing anthracnose, while for pedicle rot, *L. theobromae* and *Botryosphaeria dothidea* (Silva-Rojas and Ávila-Quezada, 2011; Fuentes-Aragón *et al*., 2018).

In other producing countries, such as Kenya, *C. gloeosporioides* and *C. boninense* were reported as causative agents of anthracnose and *Pestalotiopsis* of pedicle rot and anthracnose (Kimaru *et al*., 2018), in Israel was isolated and identified *C. aenigma*, *C. alienum*, *C. fructicola*, *C. gloeosporioides sensu stricto* (epitype), *C. karstii*, *C. nupharicola*, *C. siamense*, *C. theobromicola* and *C. perseae*, as causative agents of anthracnose (Sharma *et al*., 2017). Symptoms of postharvest anthracnose develop as light brown circular lesions, which lengthen and sink, changing to darker colors as maturation progresses (Figure 6).

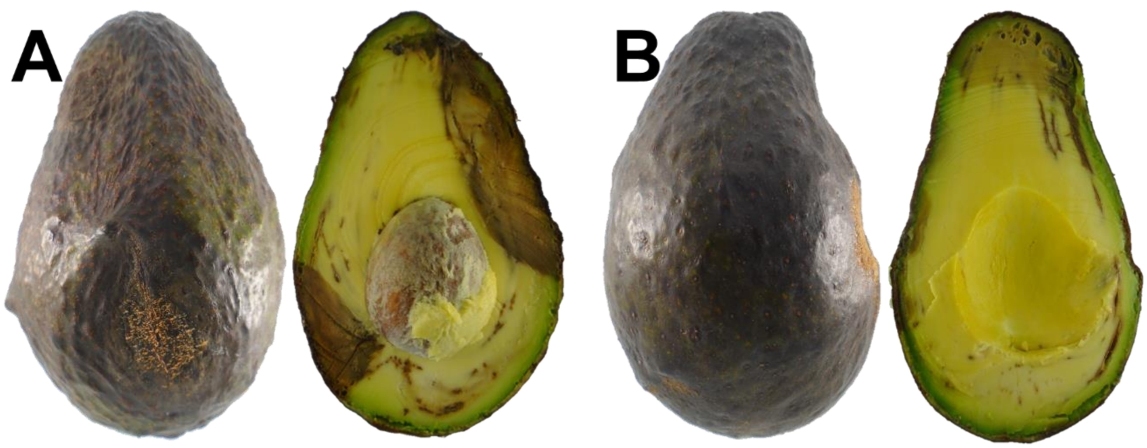

Figure 6. A) fruits with anthracnose symptoms; and B) pedicle rot caused by *Colletotrichum* spp. and *Lasiodiplodia theobromae*, respectively.

The lesions spread rapidly through the skin and pulp of the fruit, leading to rotting, which makes it difficult to observe, because the avocado ‘Hass’ changes from green to black when it reaches ripeness for consumption (Kimaru *et al*., 2018). Regarding pedicle rot, it begins in the area of insertion of the pedicel, which softens and rapidly disperses into the fruit, the pulp becomes discolored and is associated with vascular darkening (White *et al*., 2005). The severity of pedicle rot increases as storage time increases. Fast-ripening fruits are less likely to be affected by pedicle rot than slower-ripening fruits (Alama *et al*., 2006; Maftoonazad *et al*., 2007).

Latent state of *C. gloeosporioides* and *L. theobromae*

Although the symptoms of damage by *C. gloeosporioides* and *L. theobromae* are observed postharvest, infection usually occurs preharvest, from the early stages of fruit development (Figure 6). *C. gloeosporioides* can penetrate tissues by three routes: 1) natural openings (stomata, lenticels, and pedicel); 2) wounds; and 3) direct rupture of the cuticle of the fruit skin (Slippers *et al*., 2005; Prusky *et al*., 2013).

In Australia it has been observed that *C. gloeosporioides* infects the fruit and remains dormant from six months before harvest, while in Israel until three months before harvest (Everett, 2019b) in warm periods (18-27 °C) and high relative humidity and periods. In Mexico, although the infection process by *C. gloeosporioides* in the fruit has been studied (Rojo-Baez *et al.*, 2017), there is no information on the infection process in pre-harvest.

Although *L. theobromae* can penetrate the fruit through the aforementioned routes, it penetrates almost exclusively through natural openings (stomata and lenticels), when the fruit or plant is under stress or non-optimal growth conditions, such as malnutrition, water stress, temperatures over 30 °C, late harvests, excess production, lack of pruning and damage caused by hail or frost. Also, its presence in plantations established in unsuitable conditions (soil, temperature and altitude) is common, which stimulates its attack since it is an opportunistic fungus.

Once the conidia of *L. theobromae* germinate and produce the germ tube, from one or both terminations of the conidia, it grows on the surface of the fruit until it finds a natural opening. *L. theobromae* can also penetrate with and without appressorium formation, when natural openings are not available (Ki-Woo *et al.*, 1999; Slippers *et al.*, 2005; Slippers y Wingfield, 2007).

Both *C. gloeosporioides* and *L. theobromae* reach the fruit in the form of spores or conidia in order to germinate, grow and finally sporulate; for which they develop infection strategies. When the spore or conidia reaches the fruit, it performs a process of recognition, adhesion and fixation to the fruit. For direct rupture, the spore of *C. gloeosporioides* produces a germ tube, which develops an appressorium, asymmetrically polarized, with an upper dome and a complex basal pore, which synthesizes enzymes that degrade epicuticular waxes; then it generates pressure and is strongly attached to the skin cells of the fruit and begins to synthesize enzymes (cellulase, chitinase) that degrade the cell wall.

Once this step has been carried out, the appressorium maintains direct contact with the living host and produces the infection hypha, which remains in a latent state until the fruit ripens after harvest. In pre-harvest, both diseases (anthracnose and pedicle rot) are asymptomatic until the fruit begins the post-harvest maturation process, where a transition process occurs that increases until it reaches necrotrophism. All this is possible due to the fact that the defense mechanisms of the fruit are decreasing (Prusky *et al.*, 2013).

Chemical disease control

Due to the fact that the greatest loss in avocado commercialization occurs postharvest, the chemical control of pathogenic fungi is important to maintain quality during the packing, storage and transport process, until consumption, based mainly on the exclusion and eradication of the inoculum in the fruit. Although the majority of synthetic fungicides are aimed at pre-harvest, their use in post-harvest is scarce and of low efficiency. The latter may be due to the unknown pathogen that causes the disease and the existing regulations for its use in avocado for export (Cavalcante *et al.*, 2014; Everett, 2019a).

In Mexico, the National Service for Agrifood Health, Safety and Quality (SENASICA) and APEAM have drawn up a list of recommended agrochemicals for avocado cultivation, where fungicides such as Azoxystrobin, Azoxystrobin + fludioxonil, Pyraclostrobin, Copper sulfate and Thiabendazole appear for the control of anthracnose (*C. gloeosporioides*) in pre and postharvest (APEAM, 2016). Although *L. theobromae* is not included in this list, these fungicides are also used for its control. Only one complex of synthetic fungicides is authorized for postharvest application in exports to the United States of America: azoxystrobin + fludioxonil (trade name Bankit Gold®) in doses of 0.75 - 1.5 ml L^{-1} of water.

On the other hand, avocado producers, industry, regulatory agencies, the scientific community and consumers have great interest in producing and marketing fruits free of synthetic fungicide residues, therefore, it is necessary to integrate alternative systems to chemical control in pre and postharvest that are low cost, effective and with information on the dose, exposure time, time and number of applications, as well as the site and mode of action in pathogens (Kuck and Gisi, 2008; Feliziani and Romanazzi, 2016).

Conclusions

The avocado fruit is distributed, sold and consumed all over the world for its nutritional value, bioactive compounds and health benefits. This global trend will continue for years to come. Once it is harvested, the fruit begins a critical stage because the quality must be maintained; however, the presence of the pathogenic fungi that cause anthracnose and pedicle rot leads to serious economic losses.

Due to the low efficiency of the fungicides that are currently used and the regulations and prohibitions on their use in avocado importing countries, it is necessary that in Mexico other alternatives are developed, with low risk to health, low cost and at the same time effective in the control of these diseases, in such a way that they become part of the cultural practices in pre-harvest and routine stages in post-harvest. The knowledge of the protection mechanisms inherent to the fruit could allow to establish new alternatives of inhibition of *C. gloeosporioides* and *L. theobromae* that can be incorporated in any of the post-management stages.

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