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Crisis of High mountain coffee production in Mexico:
Principal contributors and further perspectives.

A thesis presented by

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Dedication

Este trabajo va dedicado a los pequeños productores de Jaltenango de la Paz, Chiapas. Quienes me permitieron entrar y conocer más sobre ese tradicional estilo de vida, lleno de sabiduría y respeto hacia la madre naturaleza. Espero que estas publicaciones puedan tener un impacto positivo a su favor, y refleje un poco la magia que conlleva ser un pequeño productor de café en México.

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Crisis of High mountain coffee production in Mexico: Principal contributors and further perspectives.

By

Nora Esther Torres Castillo

Abstract

As a researcher, we have to be able to cover a wide range of topics related to any field, from chemistry to biology. During the development of solutions, it is important to identify the roots of the problem. This can lead to the generation of knowledge from different perspectives. A perfect example is this work, which started as the study of coffee leaf rust in Mexican crops, where 70% of the total production is lost due to this disease. However, as we continued, we identify other crucial factors, not only related to coffee rust adaptation, but behind the urgent crisis of high mountain coffee in Mexico: the COVID pandemic, that has generated losses of 40% in the coffee demand, the fluctuations in the patterns of precipitation and temperature as a result of climate change since 2006 to the present time, and the lack of opportunities to access to a more competitive market, as a smallholder. Therefore, to generate an integral solution, besides politics and socio-economical reforms, we decided to take a circular economy approach. How can we create new sources of income for this sector and at the same help to reduce climate variability? Our answer, through the introduction of coffee by-products to the value chain. Mexico is the leading exporter of High mountain coffee in the world; Thus, by the generation of technology base on coffee by-products, we can tackle both problems. The examples are caffeine bio adsorbents, made of the coffee husk, or spent ground coffee, used as a substrate for bioethanol production. Even in the future, coffee by-products can serve as raw material to develop scaffolds for catalysis reactions. In the end, the principal purpose of this research was to offer alternatives to ensure the prevalence of high mountain crops, which are the basis of Mexican coffee.

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Chapter 1

Introduction

Everyone drinks coffee, whether you like it or need a cup to start your day, as 65% of the world's population (National Geographic, 2018). In fact, 2.9 million cups of coffee are consumed worldwide daily (SADER, 2020), and this makes coffee one of the most important trade commodities, and cultivars in the world. In the international market 58% of the coffee is produced by the variety *Coffea arabica*, also known as Arabica or High mountain coffee due to their growing conditions. The organoleptic properties of this variety make its coffee beans highly valuable, and the main choice for specialty roasters and tasters.

In Mexico, Arabica crops represents 85% of the coffee production, mainly produced under organic schemes, and it is distributed to EEUU, Europe, Japan, Cuba, and Canada. As result, coffee cultivation generates up to 500,000 employments in 14 federal entities, in which 95% of the coffee producers are small farmers, from lagging communities, with cultivated areas no larger than 5 hectares (SAGARPA, 2017).

Chiapas, Veracruz, and Oaxaca are responsible for 80% of the total coffee produced in the country (with 730,111 hectares sown in 2016) (SAGARPA, 2017; Avelino and Rivas, 2013). However, the biodiversity of Arabica varieties is endangered.

The national coffee production is being greatly affected by several factors as the COVID-19 Pandemic, causing a reduction in worldwide demand, coffee leaf rust, that started in 2006 and it is worsening in the past years due to climate change, and the current commercial alternatives, which are not enough to cover the small producer needs. Also, the growing demand from multinational coffee companies to supply their consumption of Robusta coffee (Aguilar, A., 2020). As result, coffee producer faced the dilemma of continuing with Arabica crops, despite the lack of support, or they should plant Robusta varieties to have an assured source of income. This represents a problem since the diversity of coffee and tropical forests

in our country hangs by a thread. Therefore, in order to generate an integral solution, besides politics and socio-economic reforms, we decided to take a circular economy approach. How we can generate new sources of income for this sector and at the same help in reduce the climate variability? Our answer, through the introduction of coffee by-products to the value chain. Mexico is the leading exporter of High mountain coffee in the world; Thus, by the generation of technology, based on coffee by-products, we can tackle both problems. The examples are caffeine bio adsorbents, made of coffee husk, or spent ground coffee, used as substrate for bioethanol production. Even in the future coffee by-products can serve as raw material to develop scaffolds for catalysis reactions. At the end, the main purpose of this research was to offer alternatives to ensure the prevalence of high mountain crops, which are the basis of the Mexican coffee.

Research Questions

- What are the main factors involve in the actual high mountain coffee crisis?
- Are climate change and the early development of *Hemileia vastatrix* correlated?
- How science can contribute to support sustainable coffee-growing communities?

Hypothesis

- *Hemileia vastatrix* is the main problem for organic coffee producers in Mexico, and its early development is directly related to climate variance, and indirectly related to the living conditions of smallholders.

Overall aim

- Study the main factors that contribute to the current coffee crisis in Mexico regarding high mountain varieties, with an emphasis in *Hemileia vastatrix* impact, as well as explore the current alternatives to support sustainable coffee-growing communities.

Specific objectives

- To identify and evaluate the principal factors that contribute to the current crisis of high mountain coffee production, based on field research.
- To study the climate dynamic and precipitation patterns over the last 14 years.
- To study the anatomical development of the disease Coffee Leaf Rust in the principal coffee varieties in Mexico (*Peñasco*, *Pacamara*, *Pache Criollo*, *Bourbon* and *Arabe*) in field and under controlled conditions.
- To evaluate the current living conditions and the opportunities of smallholders in Jaltenango, Chiapas, México.
- To assess the relevance of high mountain coffee varieties for coffee communities.
- To evaluate current alternatives for an efficient control of *Hemileia vastatrix* under a sustainable scheme.
- To evaluate alternatives to add value to coffee by-products as second source of income for smallholders to support their environmental-friendly practices.

Chapter 2

Defining the meaning of being a small coffee producer in Mexico - A case study of high mountain coffee produced in Jaltenango, Chiapas

Everyone drinks coffee, whether you like it or need a cup to start your day as 65% of the world's population (National Geographic, 2018). In fact, 2,250 million cups of coffee are consumed worldwide daily (SADER, 2020), which makes coffee one of the most important trade commodities and cultivars in the world.

In the international market, *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta) are the predominant coffee species, and their main differences relies on their taste, growing conditions and price, to mention a few.

Coffee is tasted by producers and buyers through a tasting technique known as '*coffee cupping*' which has the purpose of determining the cup profile, evaluating its taste, aroma, body, acidity, and aftertaste (Uusilehto, 2019). Regarding biochemical profile, Robusta contains twice as much caffeine and more chlorogenic acids than Arabica (Shokouh *et al.*, 2019; Jeszka-Skowron *et al.*, 2016; Ludwig *et al.*, 2014) and even though its higher caffeine content contributes to crop disease resistance (Kim *et al.*, 2010; Biratu, *et al.*, 1996), it provides an astringent and bitter taste, thus lowering the cup profile and its value on the market. Arabica, on the other hand, contains 60% more lipids and sugars than Robusta (Haines, D., 2019), which are responsible for increasing the aroma as well as the coffee's body, reducing bitterness, giving a cleaner mouth feel and rendering the beverage sweeter and floral notes, the above resulting in a high-score cup profile. Due to their organoleptic properties, Arabica coffee beans are highly valued in the international coffee market and are the beans of choice for specialty roasters and tasters. Contrary to Robusta varieties, Arabica coffee plants require specific growing conditions; they can only grow from 900 up to 2800 MASL (Schmitt, C., 2006) and therefore they are usually cultivated in the slopes of high mountains, reason why Arabica coffee beans are often called "high mountain coffee".

Arabica coffee represents 58% of the worldwide coffee production and 85% of Mexican coffee production (USDA, 2020). However, the varieties of Arabica are endangered in this country. The national coffee production is being greatly affected since the coffee rust crisis, that started in 2006, worsening in the past years due to climate change. Also, the growing demand from multinational coffee companies to supply their consumption of Robusta coffee (Aguilar, A., 2020) are key factors for a coffee producer to decide if they should deforest their coffee-rust-susceptible Arabica coffee plants and better plant Robusta varieties to have an assured source of income. This represents a problem since the diversity of coffee and tropical forests in Mexico hangs by a thread. Hence, the aim of this study is to present a global socioeconomical perspective of Mexican arabica coffee, as well as evaluate the most significant factors that may be involved in the decrease of coffee produced by smallholders in Mexico.

***Coffea Arabica*: Endangered Stem of Mexican Cultivars**

Despite the type of cultivation, it takes 3 years to have a fully developed coffee plant, from the seed to the first fruit production, and it can be productive for up to 80 years. However, based on economic lifespan, the plants do not live more than 30 years (Segura, 2017). The life cycle of the coffee plant (**Figure 1**) covers three crucial stages: Growth, production, and physiological decay (CONABIO, 2015). During the production stage the maximum crop yield is obtained, and it is the most important stage for the coffee grower (Janissen and Huynh, 2018).

This phase lasts approximately 15 – 30 years and varies by region. The regions far from the equator has an annual cycle of flowering and fruiting (Herrera and Lambot, 2017), unlike Mexico, that according to the coffee producers, can have up to 3 blooms per year, depending on water availability. Therefore, the rain season plays a crucial role because it will determine bud growth and the flowering process. During low humidity periods, the bud may fall into a dormancy interval and flowering can be interrupted. To avoid this, the plant required at least 10 mm of rainfall (Segura, 2017). In contrast, coffee fruits start their maturation during the dry

season and usually are collected by the end of the rain season (Herrera and Lambot, 2017).

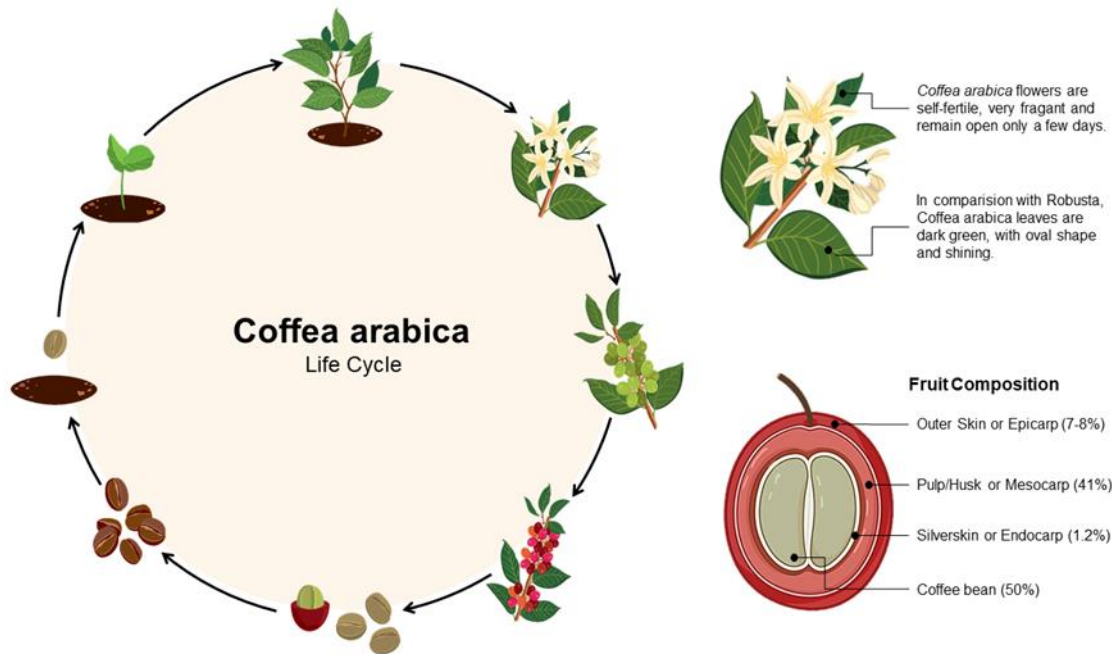


Figure 1. *Coffea arabica* life cycle. Coffee plants can be productive for up to 80 years. The growth phase can take from 4 to 7 years and is focused on plant development and differentiation (Root and aerial systems). It ends once the plant starts the germination. Eight months after the first flowering, the plant reaches its mature stage and starts the fruit production. The coffee fruit is a drupe known as coffee cherry, coffee grape or *capulín* and their brunches change from a light green to a dark red (once they are ready for harvest), except for the Catuaí variety where the mature fruit turns to amber. During the production stage the maximum crop yield is obtained, and it is the most important stage for the coffee grower. Later on, after 15 years, the plant begins to gradually decrease its production and the physiological decay takes place. The plant becomes more susceptible to changes in temperature, humidity, and disease, follow by the death of the plant (Segura Escobar, M. B., 2017; Janissen, B., & Huynh, T., 2018).

Although the life cycle seems to be the same for both species, the most notorious difference between *robusta* and *arabica* is the high susceptibility of arabica to weather variability. This is because of its specific requirements: an altitude between 600 and 2,000 meters above sea level (masl), a constant water availability (between 1,500 and 2,500 millimeters of annual rainfall), an environment without frost or prolonged drought, and with trees to provide constant shade to the crops (Janissen and Huynh, 2018; ICO, 2019). However, despite how laborious it is to produce this variety, it is the world's most demanded coffee crop, producing 60% of the total coffee (Van Der Vossen et al., 2015).

Coffea arabica, known as *high mountain coffee*, possess an allotetraploid genome (SENASICA, 2016) as a result of a cross between two wild type coffee plants and a spontaneous auto duplication of their chromosomes (Segura, 2017). Its origin lies in the high plateaux regions of Ethiopia (Abyssinia), approximately in the year 850 AD and it was domesticated in Yemen. In Mexico, the production of *C. arabica* is still based on traditional cultivation, which is focused on shade schemes. Bourbon and *Typica* (also known as Arabica, Criollo or Arabe) are the source varieties of the organic cultivars, and these lines and their offspring (**Table 1**) have been maintained for many years due to their attractive characteristics, such as vigor, high productivity and outstanding coffee quality (SENASICA, 2016). However, in the early 80s due to the rapid spread of CLR (which arrived to Mexico from Soconusco, Chiapas) and the high susceptibility of *C. arabica* (Herrera et al., 2002), it was necessary the identification of resistant genes to develop resistant crops (McCook, 2006). Focusing on the resistant grade of other coffee species (*Coffea liberica*, *C. canephora*, *C. eugenioides*, *C. stenophylla*, and *C. racemosa*), breeding strategies were applied, as introgressive breeding, using Mendelian and quantitative genetics (Rodrigues et al., 2000). This is how *Híbrido de Timor* (HdT) was discovered, which is a variety highly similar to *C. arabica*, found in Timor-Leste and possibly derived from a natural hybridization between *C. canephora* and *C. arabica*. Thus, after a decade of lines selection, HdT became the main progenitor of CLR resistant species grown today, as *Sachimor*, *Catimor*, *Costa Rica 95*, among others (Rivillas et al., 2011).

Table 1. Traditional varieties cultivated in Mexico identified by The National System of Inspection and Certification of Seeds (SNICS) and the World Coffee Research Organization.

Variety	Rust resistance (R) or tolerance (T)	Attributions	Genetic Group
<i>Typica</i>	No	One of the most important cultivars in Central America known as the genetic basis of the vast majority of the existent subtypes. One of the highest quality coffee crops (known as <i>café de Altura</i>) Low productivity and high-grown coffee (>1600 masl).	Bourbon-Typica (Typica related)

<i>Bourbon</i>	No	Genetic basis (as Typica) with an excellent cup quality and medium productivity. High-grown coffee.	Bourbon-Typica (Bourbon related)
<i>Mundo Novo</i>	No	Medium productivity with good cup quality. High-grown coffee.	Bourbon-Typica Cross between Typica x Bourbon
<i>Maragogype</i>	No	Low productivity but excellent cup quality. Stands out for the big size of the fruit. Medium/High-grown coffee (from 1000 m and up to 1600 masl).	Bourbon-Typica (Typica related) Natural Typica mutation.
<i>Pacamara</i>	No	Short plant with big fruits. Excellent cup quality and good productivity. High-grown coffee.	Bourbon-Typica Cross between Paca x Maragogype
<i>Caturra</i>	No	Short plant with good-medium cup quality and good productivity. It has two ecotypes: <i>Red Caturra</i> , and <i>Yellow Caturra</i> . High-grown coffee.	Bourbon-Typica (Bourbon related) Natural Bourbon mutation.
<i>Catuai</i>	No	Good-medium cup quality and good productivity. It has two ecotypes based on the color fruit (<i>red and yellow</i>). High-grown coffee.	Bourbon-Typica Cross between Mundo Novo x Caturra
<i>Geisha</i>	T	Exceptional cup quality, comparable to Typica, and tolerant to CLR. Medium productivity and high-grown coffee.	Local Ethiopian variety
<i>Costa Rica 95</i>	R	High productivity and adapted to warm zones and acidic soils. Low cup quality. Optimal Growth at medium heights (from 1000 to 1600 masl)	Introgression (Catimor) Cross between Timor hybrid 832/1 x Caturra
<i>Sarchimor (T 5296)</i>	R	Good productivity and cup quality but no homogeneity, and the next generations are not stable. Optimal Growth at low/medium heights (from 400 to 1600 m above sea level).	Introgression (Sarchimor) Cross between Timor hybrid CIFC 832 x Villa Sarchi
<i>Iapar 59</i>	R	Good productivity and cup quality but they do not present homogeneity, and the next generations are not stable. Optimal Growth at low/medium heights.	Introgression (Sarchimor) Cross between Timor hybrid 832/2 x Villa Sarchi
<i>Oro Azteca</i>	R	Good cup quality and high productivity. Adapted to warm zones and acidic soils. Optimal Growth at low/medium heights	Introgression (Catimor) Cross between Timor hybrid 832/1 x Caturra
<i>Anacafe 14</i>	R	Good cup quality and high productivity with big fruit size and tolerant to droughts. Optimal Growth at low/medium heights	Introgression (Catimor) Cross between (Timor hybrid 832/1 x

In Mexico, the CLR resistant varieties worked for almost 30 years until 2012, when the most serious CLR epidemic that the region has known broke out. Proving that the resistance acquired was not long-term (Chain et al., 2019). Not necessarily because of new ecotypes of HV appeared, but rather by factors such as the ecosystem conditions that favor the fungus development along with poor crop management (Cristancho et al., 2012; World Coffee Research ,2018).

Economic status of Mexican coffee and relevance of high mountain coffee

Coffee is part of the Mexican culture and the taste for it is often acquired from one generation to another, however, it is more than just tradition. In 2017, the participation of nearly 500 thousand coffee growers for coffee production was registered and taking in consideration their families, intermediary workers of the production line of coffee and the later commercialization, it is estimated that 3 million Mexicans are economically dependent of coffee in some way (CEDRSSA, 2018). The production of Mexican coffee cherry reached 900,215 tons in 2019, positioning the country as the eleventh-largest producer worldwide (SADER, 2020). Chiapas, Veracruz and Puebla are the main coffee producing states in Mexico: of the national coffee production of 2019, Chiapas contributed to the 40.9% of it, while Veracruz and Puebla contributed to the 24.2% and 16%, respectively (SADER, 2020). However, while Chiapas is dedicated to the production of Arabica coffee, Veracruz produces mostly Robusta coffee, which can be allocated to the plantations of multinational coffee companies nearby the region. This is consistent with statistics from USDA (2020), where it is shown that 85% of coffee produced in Mexico is Arabica variety and 15% Robusta.

In 2018-2019, Mexico exported 2.91 million bags of coffee, from which 59.4% corresponded to unroasted green coffee, 34.4% to soluble coffee and 6.2% to roasted and ground coffee. Approximately 57% of the exportation volume had the United States as its destination. Belgium, Spain, Germany, Canada, Cuba and Japan represented other destinations for Mexican coffee (FIRA, 2019). The United

States is, to this day, the main international market for Mexican green coffee (USDA, 2020). From January to June of the present year, 101.86 thousand tons of coffee have been exported, with a value of 427.51 million dollars, which represents an increase of the exported volume in comparison with the same period of 2019 (SADER, 2020).

Mexico is the second largest producer of organic coffee in the world, with a production volume of 350 thousand bags of 60 kilograms. In terms of exports, Mexico is the main exporter of organic coffee, sending nearly 28 thousand tons annually to European countries (CEDRSSA, 2018). In 2008, 176,108 hectares were registered as complying with organic coffee regulations, of which 55.81% were in Chiapas, mostly worked by indigenous coffee producers from disadvantaged communities (Sánchez, 2015). As a matter of fact, 85% of Mexican coffee producers are from indigenous populations, with 95% of them considered small coffee producers owning less than three hectares of land (USDA, 2020). The need to reduce the commercial intermediation of coffee and the lack of financing was what led coffee producers to create non-profit organizations aimed at facilitating access to the means of production or supplies to their constituent members – today, we know them as cooperatives. Mexico has approximately 600 cooperatives and 400 of them are from Chiapas, these being made up of 178,000 coffee producers, mostly of small producers (USDA, 2020).

However, being a coffee producer in Mexico is not easy. The biggest challenge are the fluctuant global coffee prices, with costs of production often higher than returns. During the first months of 2019, costs of production reached approximately 140 dollars per kilogram of coffee while it was sold only to 98 dollars (**Figure 2**) (USDA, 2020).

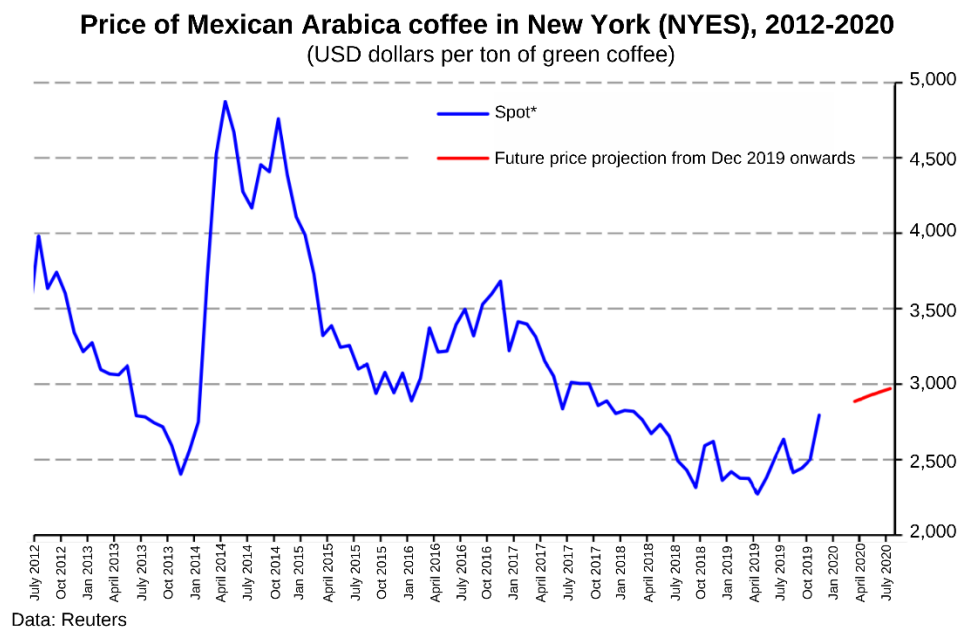


Figure 2. Price of Mexican Arabica coffee through the decade. Mexican Arabica coffee prices in the NYES from 2012 to 2019, with an estimated projection (red line) for prices from Dec 2019 to July 2020 (FIRA, 2019).

Mexican coffee producers might agree that the main problem in coffee is the market, since a kilo of coffee cherry is sold in 6 Mexican pesos but is in quintals that coffee cherry is sold internationally. A ton of coffee cherry is equal to 4 quintals. Let us assume that a quintal is worth 100 dollars, then the kilo of coffee cherry should be paid in 8 to 10 Mexican pesos, but the coffee producer still receives 6 Mexican pesos (Aguilar, 2020). “Why do I have to be tied to a market which doesn’t work?” expressed a coffee grower for Los Angeles Times (2019).

The silent crisis of *Coffea arabica* in Mexico

Mexican coffee generates foreign exchange and employment in rural environment since woman, men and young people are hired to clean, harvest and process the coffee bean (Instituto Nacional de Economía Social, 2019). From 1994 to 2004 it has generated an amount of 4,160 million dollars that represented 20.4% of the income of the sector, in addition to providing more than 300,000 direct jobs to the national economy (Villaseñor, 2004). And although at present its commercial value

has decreased, it is still an important activity economically and socially speaking (Herrera Quesada & Jan Argüello, 2013).

Currently, Mexican government is currently promoting Robusta production, producers are expressing concern that they will be forced to deforest their *Coffea arabica* already productive trees given that Robusta trees require full sun. It has been demonstrated that *C. arabica* requires lower solar incidence than other species to maintain coffee productivity (Gichuru *et al.*, 2013), which allows coffee producers to maintain a shade grown scheme; the system of coffee plantations in Mexico is mainly under shade, which promotes the conservation of biodiversity such as flora, fauna and aquifers as well as carbon sequestration (Instituto Nacional de Economía Social, 2019).

The support of the Mexican government to allow multinational companies to open new coffee plants and to distribute Robusta varieties used for the making of their soluble coffee among coffee producers (Aguilar, 2020), along with the fluctuating and decreasing prices of Arabica in the international market, may constitute a key factor for coffee growers to accept replacing their Arabica cultivars for Robusta as the latter is coffee rust resistant, a coffee disease that threatens the production of *Coffea arabica*.

Trade alternatives for small coffee producers in Mexico

The main goal of every peasant will be to achieve autonomy; Thus, they are only partially integrated into markets. There are commonly covered by combining production for the market with subsistence cultivation and/or other varied forms and degrees of dependence on off-farm work. Most of southeast Mexico's coffee producers are indigenous and, in addition to exploitative working conditions, also suffer racial forms of oppression in the diverse local, national, and international labor markets they enter (Henderson, 2019). By understanding the ways in which struggles for autonomy from and within the market articulate and adapt to changing market conditions is important analytically and for its practical significance.

In addition, the new market trends can be a problem because it separates consumers from smallholders, and the new schemes focused on fair trade and environmental consciousness (**Figure 3**).

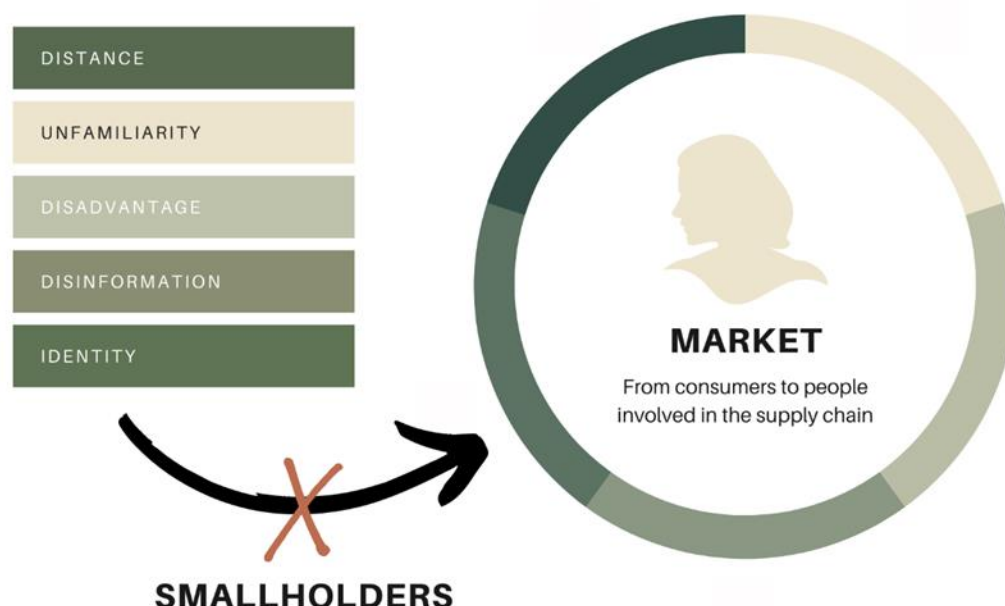


Figure 3. Main barriers between smallholders and the current market, based on Stoknes P. E. diagram of five barriers. Exists barriers that do not allow the entrance of small coffee producers to current markets. One of them is Distance, where people struggle to get involved with the current situation of small producers, because they perceive it as something distant, be it temporally, geographically, or socially. Then, unfamiliarity.

The traditional schemes possess at least 4 intermediaries, generating a revenue of just 0.4% for the producer (**Figure 3**). However, due to growing difficulties, peasants may be forced to make a trade-off, sacrificing certain elements of their autonomy to increase others, aiming to secure their long-term access to the land (Henderson, 2019).

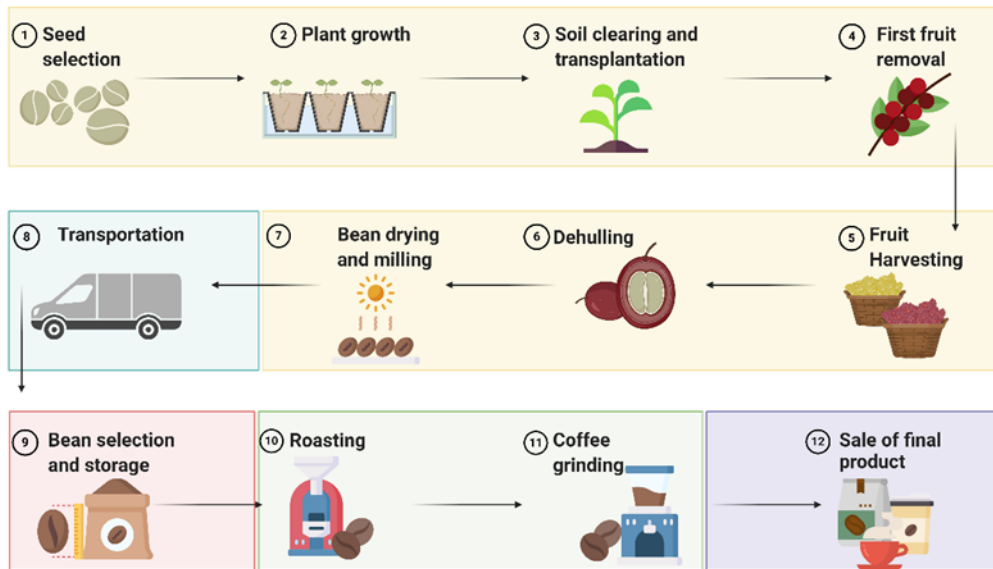
Farmers in developing countries are exposed to many risks, as price volatility and declining prices. Also, due to weather conditions, the risk of pests and diseases or unforeseen events such as natural disasters, farmer ill-health or political turmoil. Poorer farmers respond to risks by lowering investment and yields, resulting in more secure but lower incomes (Ellis, 1998). This may perpetuate inequalities by creating poverty traps where marginalized farmers are unable to improve their situation.

For this reasons, Fair-Trade born as an alternative to support smallholders (**Figure 4**); And since, no other global actor has attempted to increase agricultural commodity prices for developing-country farmers after the demise of commodity agreements and their production quotas in the 1980s and 1990s (Gilbert, 1996).

Fair Trade involves more than providing higher prices to farmers. It aims to improve value chains, to include developing-country farmers and workers in a dialogue on conditions of production, it allows the empowerment of marginalized farmers and their organizations through capacity-building; Also, this strategy can provide credits and secure democratic decision-making in producer organizations.

Fair-Trade sets standards for environmental and labor conditions of production. In the case of some products such as coffee, it favors small scale production by certifying only co-operatives of small-scale farmers. Latin America is the center of Fair-Trade coffee production, where Mexico has been the dominant player (Murray, Raynolds, & Taylor, 2006).

Case A: Coffee production under the traditional scheme



Case B: Coffee production under the management of a cooperative

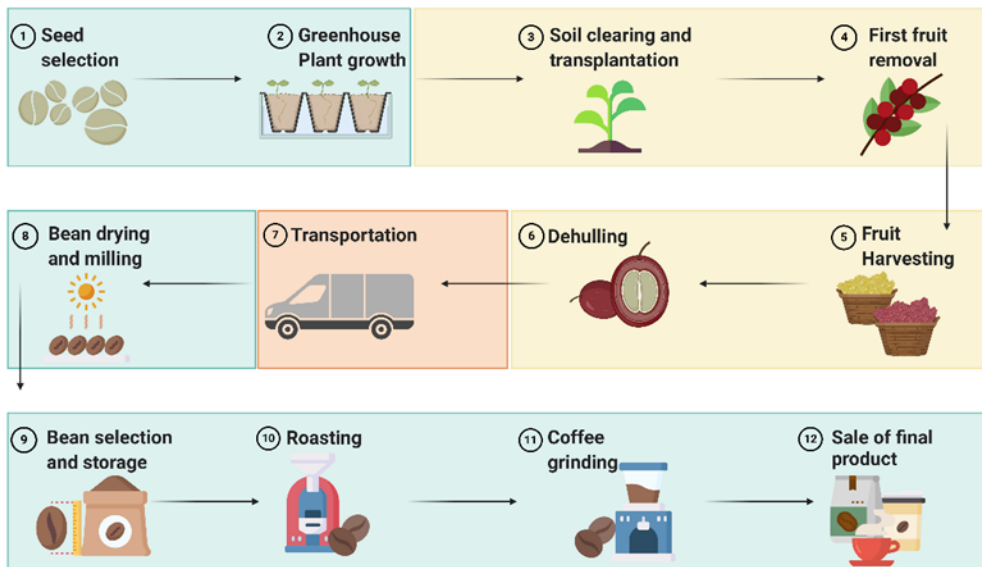


Figure 4. Comparative analysis of traditional production and under the management of a cooperative.

In the traditional scheme, at least 4 intermediaries exist. Under this scheme, the coffee producers make all the hard work, from the seed selection to the bean preparation to sell it as green gold, and they received only the 0.4% of the total profits, which is not even close to enough to cover all the production expenses and the work staff recruitment. On the other hand, the cooperatives, which in the majority are under fair trade schemes, they offered a direct deal with the smallholders. Normally the only intermediary is the transport. The cooperatives

manage from the bean drying and milling to the sale of the final product, both national and international shipping. Other important characteristic of cooperatives is that they do the seed selection and have their own greenhouse to produce young plants (*piloncillos*) and organic fertilizers, as humic acid. These are provided to the smallholders, which represents important savings for them.

Small coffee producers facing COVID-19

Due to the COVID-19 pandemic, many countries are taking measures to control the spread of the virus. Even though the health aspects of the pandemic have not affected rural areas like urban centers, it has affected the production of vegetables and fruits, which are the basis for smallholder's survival (FAO., 2020). Since the beginning of the pandemic, many agriculture markets have been affected, and coffee production was no exception. Due to the current state of urgency, priorities have changed, and the economy has slowed down. Since March 2020, when Coronavirus was announced a worldwide pandemic by the World Health Organization (WHO), coffee costs have been highly unstable, mainly because of flexibly chain interruptions. At the end of June, the ICO composite marker fell beneath the 100 US-cents/lb mark. It is obvious that Coronavirus pandemic is and will be an enormous additional challenge to the worldwide coffee area that has encountered a delayed time of low costs (ICO, 2020).

As Daley, B., (2020) explains:

“the Coffee sales has been hit hard by COVID-19 – particularly for producers like my family who are dedicated to cultivating high-quality coffee for export. They are not used to selling coffee within the country and are not diversified into other agricultural products. Due to the pandemic, the government has imposed restrictions that have prevented millions of sacks of coffee from being exported”

The demand for coffee is still strong. However, the problem is who will provide that coffee if the dynamic of shopping has changed. Currently, e-commerce and online sales are the principal alternative to obtain the products of interests. Unfortunately, the vast majority of smallholders do not have access to basic services, including internet. Thus, this new type of economical dynamic is affecting hardly this sector (WHO., 2020; ICO., 2020).

Prior to COVID-19, the system had already failed to create resilient, prosperous small-scale producers. However, this can also bring us a new alternative for our small producers. Our hope is that rather than returning to a pre-pandemic normalcy, or worse, COVID-19 can be the impetus to make the system more just and productive. It has made evident the enormous difference between big companies and small producers. Therefore, this opens the possibility to offered new ways to commercialize their coffee and change their actual structure with the correct support. There is no inherent trade-off between a resilient, productive smallholder system and one that is fair. Indeed, the Global Commission on Adaptation states that with governments investing more than USD10 trillion in crisis relief in 2020 alone, COVID-19 is an impetus for smallholder systems to “get on a new path, one that makes human society more resilient, more equitable, healthier, and stronger” (Guido, Knudson, & Rhiney, 2020).

Jaltenango - Cradle of the high mountain coffee in Mexico

The Sustainable Rural City of Jaltenango (SRCJ) was founded in 2012, located in the municipality of Angel Albino Corzo, where around 2,813 people from 15 communities, including Monterrey and Plan de Libertad, were relocated (Torres *et al.*, 2016). Jaltenango is located near to El Triunfo Biosphere Reserve. The SRCJ is an urban-rural space (rural zone in which the possibility of taking the benefits of the city is sought), it has a territorial extension of 80 hectares, its altitude is 614 MASL and 625 houses are registered (OPS México, 2016). Coffee from Jaltenango city is known for its superior quality, precisely because its community consists mostly of families from coffee-growing regions. Coffee growers in Jaltenango depend heavily on the income generated from their cultivars, therefore they frequently must travel to their working lands since SRCJ does not have an optimal altitude for the growth of Arabica coffee varieties. Since the coffee rust crisis, crop losses have greatly affected the volume of national coffee cherry production (SIAP, 2019).

Most coffee growers in Jaltenango are dedicated to the production of organic coffee and therefore do not use chemical products on their coffee plantations.

Organic producers constantly use other natural methods to care for their crops and ensure good production, such is the case of shade-grown coffee plantations and the addition of minerals to enrich the soil. The coffee producers themselves plant endemic trees in the region so that coffee crops grow under the trees' shade, which brings benefits to the crops such as preventing the transmission of fungal diseases (i.e., rust), inducing mineral recycling and concentration of phosphorus and nitrogen in the soil, among others – this practice increases the biodiversity of flora and fauna (Torres *et al.*, 2020). Nonetheless, due to the current problems, some producers have opted to abandon their organic practices in favor of monocultures, which has encouraged the felling of trees and loss of biodiversity. At the same time, given that coffee growers are not generating the same amount of income in comparison with previous years due to low production caused by coffee rust, it has become harder for them to afford the costs of planting and harvesting, and have no possibility of obtaining rust control agents. Some of them can no longer live solely on coffee and others choose to migrate to countries such as the United States in order to sustain their families.

Chapter 3

Studying the resilience of *Hemileia vastatrix* under current climate conditions and control strategies.

Climate change can be defined as the unusual weather variability on Earth that has persisted over the years, as result of the excessive increase of greenhouse gas emissions, mainly produced by human activities, according to the Intergovernmental Panel on Climate Change (IPCC) (Cuestas, 2018). The main concerns due to climate change are the accelerated rate of biodiversity loss and the alteration of wildlife distribution (O'Connor et al., 2020), which translates into the reduction of available spaces for agriculture, and therefore, food shortages (Rice, 2018). But what is the connection between this phenomenon and the organic cultivars in Mexico? The answer is the current coffee production crisis that has severely impacted the economy of the smallholders from indigenous communities, which are the principal producers in the country (Camargo, 2010).

Currently, Mexico is the leading producer of organic coffee in the world (Chain et al., 2019); however, it has been struggling since 2006 to maintain this position due to the rapid spread of the fungus *Hemileia vastatrix* (HV), the agent that causes the disease known as coffee leaf rust (CLR) (Libert and Paz, 2018). In 2012, the temperature of the mountainous areas in the south of the country (above 1200 masl) increased by 1°C, where primarily coffee crops are cultivated (SMN, 2020; Wintgens, 2004). In the same year, an earlier and aggressive outbreak of HV caused the loss of 50% of the total national coffee produced (Cuesta, 2018; Talhinhos et al., 2017; Wintgens, 2004). This loss was the trigger that started the crisis and to date, the country has not been able to recover its production rates, despite the efforts of the government and farmers (Libert and Paz, 2018; Talhinhos et al., 2017). The current situation has made evident the need for research in Mexico on three crucial aspects regarding the disease: The pathogen itself, its interaction with the native coffee varieties under organic schemes, and the environmental factors involved in the HV development. Thereby, to develop action

measures and regulations to support the small producers by controlling the increasing propagation of CLR (Camargo, 2010; Libert and Paz, 2018).

Therefore, this chapter discusses the contribution of climate change effects in the early development of CLR in Mexico, as well as the current actions that are taken around the world to control HV rapid proliferation and preserve the vulnerable organic crops.

Fieldwork and Bibliographic Research

Intending to generate an overview of the situation in Mexico, 267 journalistic notes from national (El Universal, El País, El Reforma, Milenio, Excelsior) and local newspapers (from Chiapas, Oaxaca, and Veracruz) were analyzed. The keyword for the search was the coffee crisis, from 2006 to 2019.

Subsequently, a field investigation was carried out in June of 2019 and February of 2020, both in Jaltenango, Chiapas, one of the coffee-growing regions most affected by rust. For the research, we interviewed the members and directors of the organizations Triunfo Verde (<http://www.triunfoverde.org/>), which is composed of 448 productive families, and CESMACH (<https://cafecesmach.mx/>), integrated by 619 smallholders.

The bibliographic study was initially carried out in international databases such as Scopus, Web of Science, ScienceDirect, Redalyc, and Scielo in January of 2020. A combination of keywords was used as criteria in any topic, title, or text words. The keywords used were: i) *Hemileia vastatrix*, ii) Coffee Leaf Rust, iii) climate change and, iv) agriculture. In total, 1010 candidate publications were obtained, of which only 184 were preserved. The criteria used to classify and summarize the selected articles were the following: i) Biotic factors related to HV, ii) Seeding methods and HV, v) control strategies, vi) Flora and fauna in coffee agroforestry systems, and vii) Effects of climate change on agriculture.

Due to the review approach, focused on Mexico, it was necessary to make a second bibliographic compilation. For this, the databases of the country's government entities (SAGARPA, CONABIO, and SENISICA) were consulted on February 2nd of 2020. In total, 52 technical sheets were compiled, where the

selection criteria were: i) *Hemileia vastatrix*, ii) Organic cultivation in Mexico, iii) Organic control and iv) Climate change and agriculture. However, the last statement did not return any search results. Regarding the climate analysis, the average temperature reports, and precipitation indexes of the country, retrieved from the National Meteorological System, were consulted, from 1985 to 2019. From which, the data corresponding to the states of Chiapas, Oaxaca, and Veracruz were analyzed and plotted (data available in supplementary information). Finally, for the same time interval, the coffee production reports provided by the International Coffee Organization (ICO) were consulted.

Plant Selection and monitoring the infection process under controlled conditions

We select five varieties (two years old plants) in the field from the coffee communities located in Jaltenango, Chiapas. First, two highly susceptible (*Typica* and *Bourbon*) and, three suspected to possess a certain level of resistance (*Pacamara*, *Pache Criollo* and *Peñasco*). Those plant were translated to a greenhouse, in order to study their interaction with *Hemileia vastatrix* under controlled conditions (the same temperature, soil and nutrition state). We selected fours plants of each variety for the control group and four for the experimental group (n=8, for each variety). Then, we perform the inoculation experiment with *H. vastatrix* uredospores retrieved from Jaltenango, Chiapas. Once the infection started, we collect the leaves from both groups after inoculation (3, 15, and 30 days post infection). However, only the most contrasting varieties (and based also in their commercial relevance) were chosen for further experimentation (**Annex Figure 1; Annex Figure 2**).

Hemileia vastatrix and Organic crops in Mexico

The fungus HV, belonging to the family *Chaconiaceae* and the division *Basidiomycota*, is a hemicyclic microorganism with an urediniosporic life cycle (**Figure 5**). It only infects the genus *Coffea*, with different levels of severity (CONABIO, 2015; Rodrigues et al., 2000; Koutouleas et al., 2019), and it prefers

C. arabica instead of *Robusta*. It was first described in 1869 in eastern Africa and Ceylon. Since then, it has been widely studied in Asia, Africa, and America due to the severe production losses generated by CLR (Glazebrook, 2005).

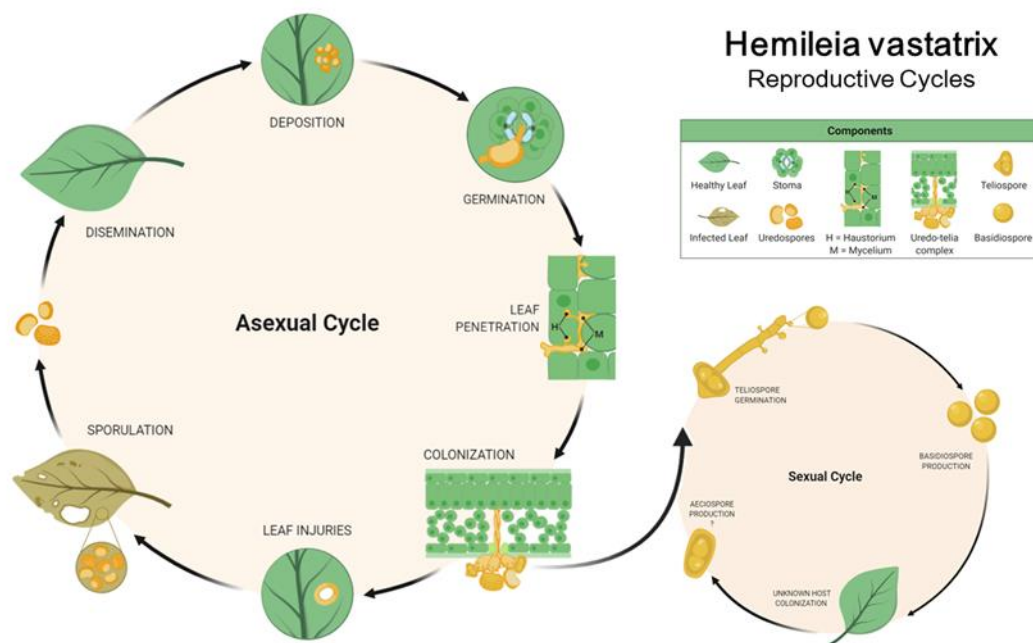


Figure 5. Disease cycle of the Coffee Leaf Rust by *Hemileia vastatrix* in coffee leaves. The infection cycle begins in the dissemination phase when the asexual spore (uredospore) is deposited on healthy coffee leaves. Subsequently, the germination stage begins when the uredospore comes into contact with the stomata located on the lower side of the leaf and begins to generate an appressorium, which gives rise to a sharp hypha that allows the fungus to pass into the internal tissue. At this point, the infectious process has begun. Nonetheless, the fungus continues to grow at the expense of its own reserves. During penetration, the mycelium invades the stomata subsidiary cells and begins to differentiate. Thus, generating the haustorium, specialized structure that absorbs the nutrients necessary for the growth of the fungus. For the first time there is a trophic relationship between fungus-host, that is, the infection has been formally established. In the colonization stage, the mycelium invades the sub-somatic chambers, and the uredo-telia complex begins with the production of sexual spores (basidiospores), and asexual (uredospores) spores. Some of them emerge abroad (formation of Soros) and it is the first lesions of the infection appear. The sporulation stage of the onset and in each lesion can find an estimated 300,000 spores, which require water as the main form of dispersion to begin the new infectious cycles (Avelino, J. and Rivas, G., 2013; Talhinhos, P. et al., 2017).

Currently, 45 pathogenic races of HV are known determined by the type of virulence factor possessed (from v1 to v9 and in combination) and these factors reacts with resistant genes from the coffee plants (SH). Thus, over the years

different ecotypes has been discovered, even races with the capacity to live in temperatures of 15°C, making them suitable pathogens to infect high-grown *Coffea* varieties. In Central and South America, the most common races are II (v5), I ((v2 v5), III (v1, v5), XV (v4, v5), and X (v1, v4, v5), and infection studies conclude that the more virulence factors they have, the less aggressive they are, due to their longer latency periods and reduced sporulation capacity (Chain et al., 2019). In Mexico, the ecotypes belonging to race II are the most common in coffee crops from Chiapas, Veracruz, Oaxaca, Puebla, Guerrero, Hidalgo, Nayarit, San Luis Potosí, Jalisco, Colima, Tabasco, Estado de México, Querétaro, Morelos, and Michoacán. Regarding HV proliferation, Chiapas is the most affected region, with losses up to 80% of total production (Hindorf and Omondi, 2015). Even though the fungus has always been present in our coffee cultivars, the aggressiveness of HV has been risen over the years (up to 30%), and the atypical outbreaks are being more common every year. For instance, in the year 2012 the HV was detected for the first time at 1750 masl in Chiapas, Mexico (Hindorf and Omondi, 2015). Besides the height, currently, the farmers found HV growths since June (when the normal months are From November to February), due to atypical temperature and precipitation patterns. Hence, it is crucial to understand in detail the mechanism of infection and the interaction between the host plant and the fungus, as well as the factors involved in the increasing pathogenicity of HV.

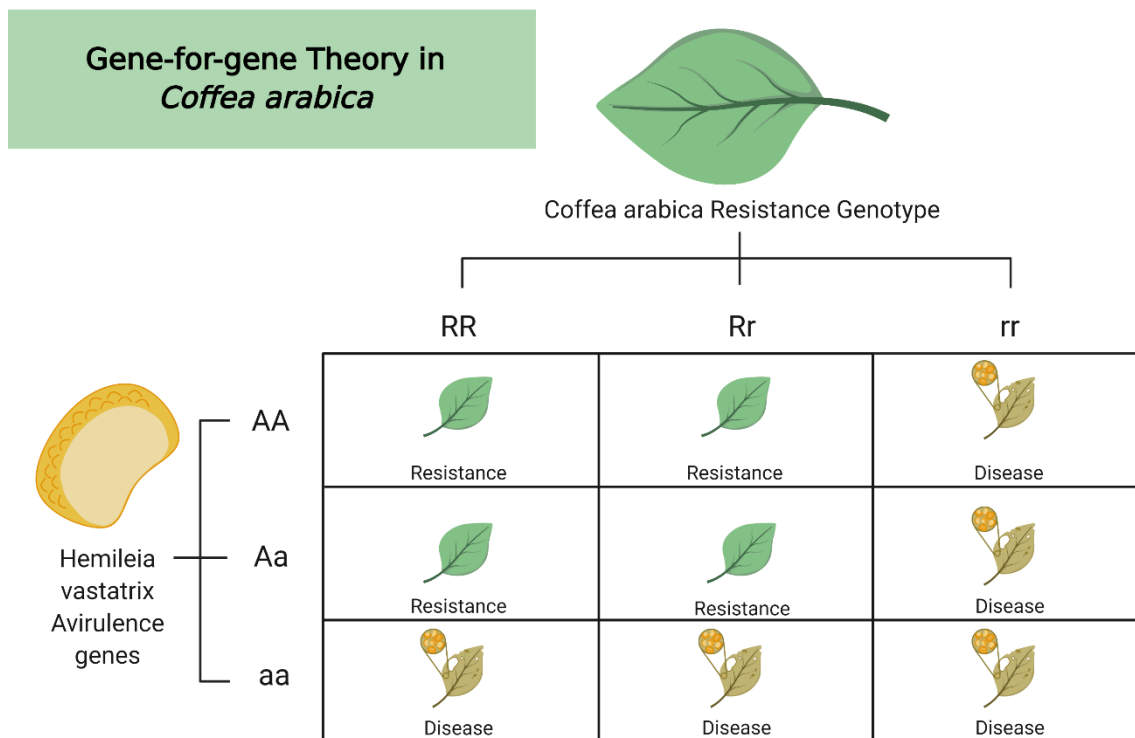


Figure 6. Scheme based on the Gene-for-gene theory of H. H. Flor showing possible genotypes in the coffee leaf rust (CLR) disease between *Coffea arabica* and *Hemileia vastatrix*. AA and RR represent the homozygous dominant genotype, while Aa and Rr are the dominant heterozygous genotypes. *Coffea sp.* presents monogenic resistance (also known as race-specific or vertical resistance) against CLR, whereas the grade of resistance can be quantified, and it can vary, depending on the expression of one or more genes (Flor, H. H., 1954; Segura Escobar, M. B., 2017).

Coffea sp. presents monogenic resistance (also known as race-specific or vertical resistance) (**Figure 6**) against CLR, whereas the grade of resistance can be quantified, and it can vary, depending on the expression of one or more genes [34]. However, the CLR resistance not always is complete. Due to the potent adaptive capacity of HV, mutation of the fungus on avirulence genes is increasingly common. When the mutation occurs, the avirulence gene is transformed into a virulence gene and the resistance host becomes susceptible, leading to the activation of the hypersensitive response as first defense mechanism (**Figure 7**). This phenomenon is known as incomplete resistance (Segura Escobar, M. B., 2017).

HV has co-evolved to modify the metabolism of the vulnerable phenotypes, according to its needs. This mechanism involves the release of effector proteins that are able to inhibit the host's immune system (Talhinhas, P. et al., 2017).

Regarding the specific genes expressed during CLR infection, at the moment, 402 annotated genes have been discovered, with crucial functionality in regulation processes as Metabolism, protein expression, defense mechanism, signaling, and the vast majority with an unknown activity (Fernandez, D., et al, 2004).

In the early stages of CLR, the plant suffers some changes as the decrement of hydrolases and oxidases, due to the lack of sugars and peptides. Therefore, to control the infection progression, alternative mechanisms are activated, as the increment of defense-like proteins to control the severity of HV. The up-regulation of hypersensitive response matches sequences that encode for proteins as phenylalanine ammonia lyases, peroxidases, superoxide dismutase chitinases, and β -1,3-glucanases, and heat shock 70 proteins, cytochromes P450, receptor kinases, AP2 domain, and WRKY transcription factors (Guerra-Guimarães, L. et al., 2009; Talhinhas, P. et al., 2017). This starts 24 – 48 hours after HV uredospores arrived. The proteins encoded are responsible of a defense mechanism based on increase of secondary metabolites, where chlorogenic acids are the most abundant phenolic compounds (Gaascht F., et al, 2015). In the first peak of the infection, before HV colonization, an early accumulation of phenolic compounds starts, leading to cell death to avoid the spread. If this accumulation of phenolic compound it is not enough, then, as a second type of protection, the plant will start with a later accumulation of phenols and the lignification of the host cell walls (Silva, M.C., et al, 2002; Toniutti L., et al, 2017).

However, in some cases, despite the effort of *Coffea sp.* to control the infection, these defense mechanisms occur late, such as to effectively prevent fungus growth and sporulation (Silva, M. C. et al., 2006; Guerra-Guimarães, L. et al., 2009).

Based on the interaction between the virulence genes from *H. vastatrix* (HV) and resistance genes from *Coffea arabica*. The virulence gene v5 comes from HV race II, the most common variety in Mexico. A susceptible host (A) is produced due to a compatible Plant-Pathogen interaction because of the perfect match between the

virulence gene from *Hemileia vastatrix* (v5) and the resistance gene from *C. arabica* (SH5). As a result, receptors on the cell membrane in the *C. arabica* are activated by the pathogen elicitors, leading to the activation of the hypersensitive response, as a defense mechanism against coffee leaf rust (CLR).

In contrast, a complete resistance is present (B) because of the genetic incompatibility between *Hemileia vastatrix* virulence gene (v1) and resistance gene from *C. arabica* (SH5). Hence, the Plant-Pathogen interaction is not produced, and the infection do not proliferate (Talhinhas, P. et al., 2017).

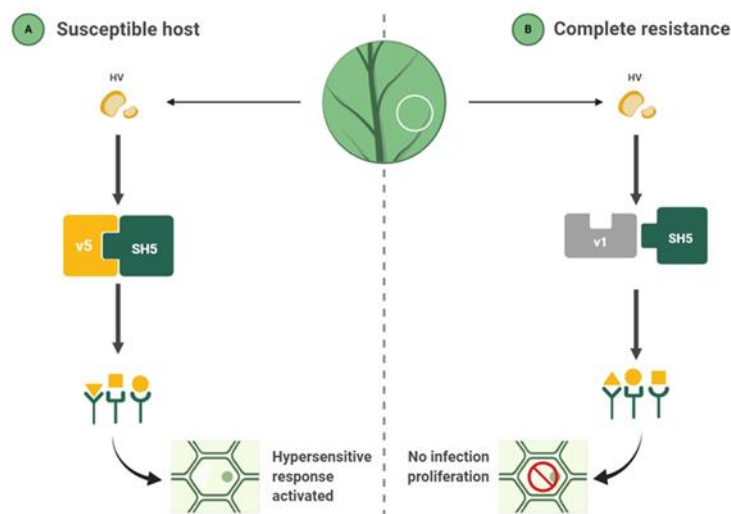


Figure 7. Development of complete resistance and susceptible phenotypes based on the interaction between the virulence genes from *H. vastatrix* (HV) and resistance genes from *Coffea arabica*. The virulence gene v5 comes from HV race II, the most common variety in Mexico. A susceptible host (A) is produced due to a compatible Plant-Pathogen interaction because of the perfect match between the virulence gene from *Hemileia vastatrix* (v5) and the resistance gene from *C. arabica* (SH5). As a result, receptors on the cell membrane in the *C. arabica* are activated by the pathogen elicitors, leading to the activation of the hypersensitive response, as a defense mechanism against coffee leaf rust (CLR). In contrast, a complete resistance is present (B) because of the genetic incompatibility between *Hemileia vastatrix* virulence gene (v1) and resistance gene from *C. arabica* (SH5). Hence, the Plant-Pathogen interaction is not produced, and the infection do not proliferate (Talhinhas, P. et al., 2017).

Global warming and Coffee Rust in Mexico a correlated interaction?

Climate change is the biggest threat to mankind and is the cause of nearly 0.4 million deaths a year worldwide, costing the world more than US\$ 1.2 trillion. It is a

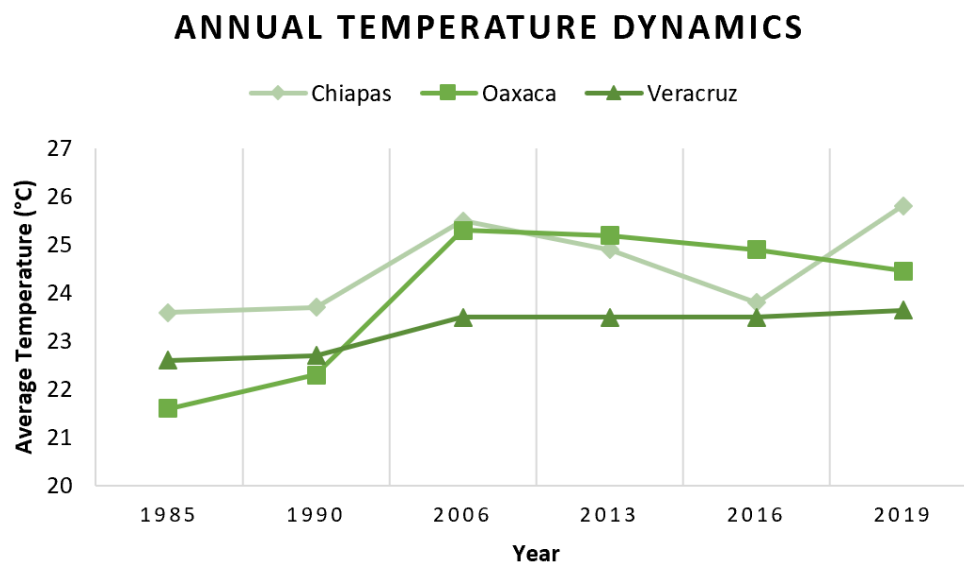
reality for most of the food crops and coffee cultivars are not the exception (ICO, 2019). As in any ecosystem, the principal factors to measure the impact of global warming are temperature, relative humidity, carbon dioxide concentration and precipitation. Thus, warmer conditions have been the key for early outbreaks of leaf rust; Mainly because winters are less cold, increasing the amount of inoculum for further HV infections in the spring (Gautam et al., 2013).

It is not new that global warming is affecting our agriculture. Studies have demonstrated an 0.74°C average global increment in temperature in the last 100 years. Likewise, atmospheric carbon dioxide (CO₂) concentration, which has risen from 280 ppm in 1750 to 400 ppm in 2013 (Villarreal, 2018). As a consequence, around the world, crucial characteristics in coffee cultivars are being affected, as crop yield and quality, due to the evident dependence of coffee crops with their environment (directly by the negative effects of the greenhouse phenomenon, and indirectly, through diseases and pests as CLR).

First of all, to understand the impact of climate variability, it is important to know about the coffee crops distribution around the world. Coffee crops can be found in a traditional Polyculture, Commercial Polyculture, and Monocultures. Each of the forms of cultivation brings with it advantages and disadvantages. For example, traditional polycultures and rustic crops, reduce leaf perspiration, the speed of night heating and daytime warming, thus the plant suffers less stress caused by abrupt changes in temperature. From the environmental point of view, these systems allow the conservation of a large number of plants, birds, and animals and maintain the native ecosystems. In contrast, the commercial polyculture only manages to retain the species correctly associated with the crops that generate the monetary utilities for coffee growers. Monoculture and commercial polyculture are the most productive in the short term. However, they are the most susceptible crops to pests because they offer a more conducive environment for pathogen growth (Janissen and Huynh, 2018).

Thereby, unlike other countries, Mexico has a unique way of production, where producers are mainly indigenous communities located in forests, and the majority of the crops are traditional shaded polycultures based on sustainable approaches

(Villareal, 2018). Those ancient practices are the reflection of the strong connection between indigenous communities and nature, and the knowledge related to the coffee ecosystem has been inherited for generations. In spite of the contribution to the environment, this way of farming has the disadvantage of being completely dependent on consistent rainfall, temperate weather, and predictable season cycles (Henderson, 2019). For this reason, since 1981 (the first report of CLR), coffee growers along with the government have been working together with the aim to adapt arabica cultivars (Murray, 2018); however, it is becoming increasingly difficult since the optimal germination temperature of HV is above 24°C and the optimal cultivation temperature of *Coffee arabica* ranges between 18 and 21°C. This rapid adaptability has made useless the efforts to control the epidemic and production losses of at least 30% are becoming more frequent (Medina et al., 2016).



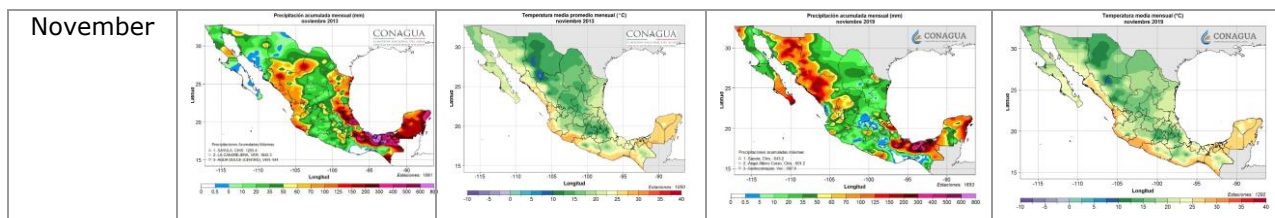
Graph 1. Tendency of the average annual temperature of the principal coffee production states in Mexico. Chiapas, Oaxaca, and Veracruz are the states evaluated from 1985 to 2019. Since 2006, it is observed a dramatic increment in the temperature (over the average 24-25°C) and remains above average nowadays. Data retrieved from the National Meteorological Service of Mexico (SMN).

The empirical period with the greatest impact, recorded to date in Mexico, occurred between 2008 and 2013 (SAGARPA, 2017), and according to Bebber *et al.* climate change was not entirely responsible. Other crucial factors were poor pest management and crop neglect (). However, one of the main effects of climate

change has been the increase of natural phenomena (Gautam et al., 2013). On this regard, coffee growers based on the high-cultivation area in Chiapas (from 800 to 1800 masl) argue that crops are highly productive when environmental conditions are more stable, in specific the temperature. This statement was the basis for a data analysis focused on temperature dynamics, from 1985 to 2018 (**Graph 1 and Table 2**). Thus, the study showed a coincidence between the stages of greater fungus prevalence and dispersion in the country, and drastic temperature fluctuations, presented since 2006, where the average annual temperature exceeded the 24°C. As consequence, the survival, infection, and dispersion rate of HV increased (Hindorf and Omondi,2011).

Table 2. Overview of precipitation behavior and average temperature in Mexico. Comparison between 2013 and 2019. In the precipitation maps range established goes from the 0.5 – 5 mm (light green) to the 600 – 800 mm (lavender). In the case of the temperature, the limit values are established from 5°C (Light blue) to 40°C (Crimson).

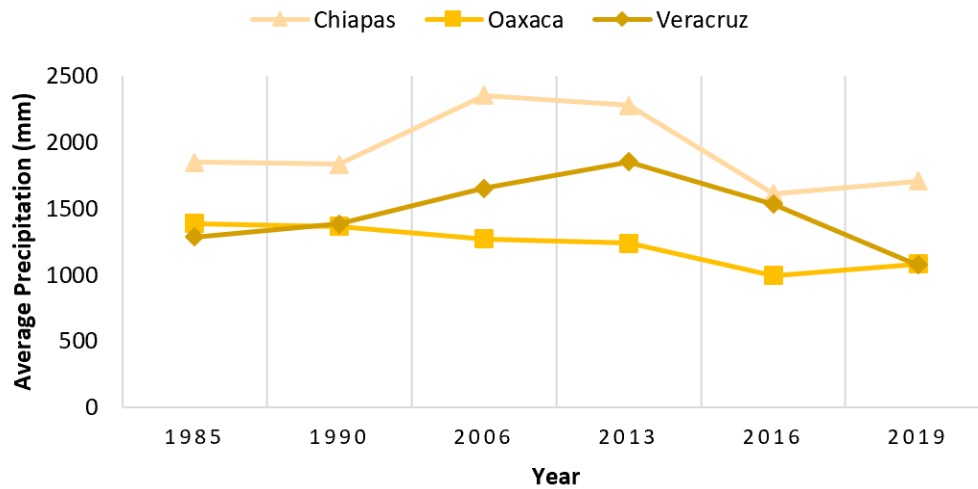
Year	2013		2019	
	Precipitation	Temperature	Precipitation	Temperature
August				
September				
October				



*Comparison between 2013, the year with the lowest coffee fruit production, after the CLR crisis, and the current year 2019. It is focused on the months with more CLR incidence, which is the stage of fruit development (October and November). However, August and September were added due to the reported cases in the last decade, where earlier CLR development had appeared. Data retrieved from the National Meteorological Service of Mexico (SMN).

According to climate projection models, La Sierra Madre de Chiapas, one of the most important coffee-producing areas in México, will present a reduction of 97% of the areas suitable for coffee growth (from 265,400 ha to 6000 ha) by 2050 (Gautam et al., 2013). Also, a study based on the IPCC (Intergovernmental Panel on Climate Change) climate change scenarios A2 and B1, made in Brazil for the years 2020, 2050 and 2080 show that as the time progress and under the current non-sustainable agriculture schemes, the incubation period of HV will be reduced, as well as the infection period and symptom expression (Stepp, 2018). During the different stages of coffee production (consisting of induction to flowering, flowering, fruit production, and ripening), the dynamics of temperature and rainfall remained stable from 1985 to 2005 approximately. However, since 2006 the precipitation pattern changed, and the futures scenarios show that the rainfalls will be more aggressive, but the rainy season will be shorter as time pass by (**Graph 2 and Table 2**). By 2050, if this variability continues, the optimal height suitable for coffee systems will be above 1600 masl instead of 1200 masl (Stepp, 2018). According to Stepp J. R. by 2030, it is expected an increase of 1.6°C in the South of the country, under a medium-high emission scenario (Eakin et al., 2018).

ANNUAL PRECIPITATION DYNAMICS



Graph 2. Tendency of the annual precipitation of the principal coffee production states in Mexico. The states evaluated are Chiapas, Oaxaca, and Veracruz in the period of time from 1985 to 2019. In 2006, it is observed a dramatic increment in the precipitation and remains above average nowadays. Data retrieved from the National Meteorological Service of Mexico (SMN).

Under this panorama, coffee production shows an uncertain future, owing to the current frequency of natural phenomena, such as landslides caused by floods or hurricanes, as well as longer heat waves that affects water storage in the soils, according to the small owners of Oaxaca, Veracruz, and Chiapas. However, smallholders are still persistent in their beliefs about sustainable agriculture despite the negatives impacts of global warming. This reality just shows the potential opportunity for proactive domestic policy to enhance both domestic food security and national-level resilience (Rice, 2018), and even though climate prediction is not an exact science, the data retrieved by these dynamic models can contribute to preventing more losses regarding coffee production.

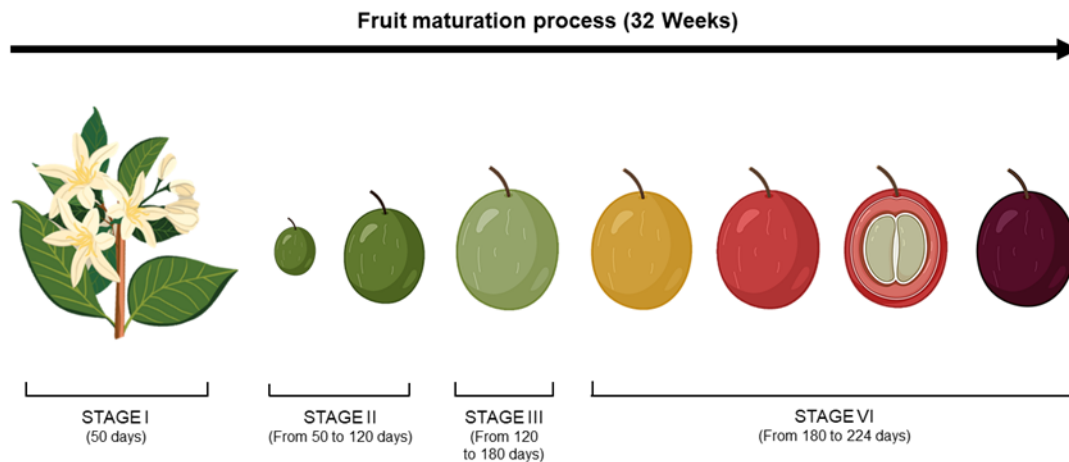
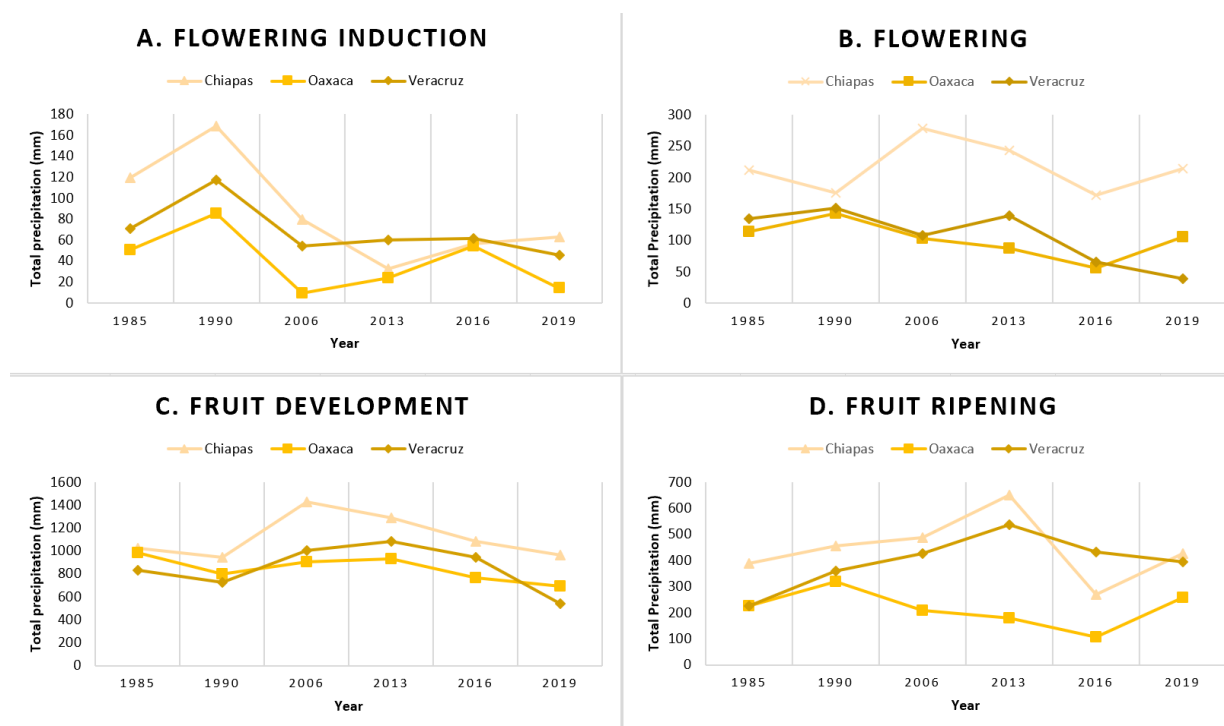


Figure 8. Coffee fruit maturation process for the variety Typica. The maturation process can take 32 weeks approximately. From the pollinized flower to the mature coffee cherry, the fruit passed for 4 major stages. The stage I takes place once the flower is opened and ready to be pollinized. When this occurs then the flower starts a slow transformation, leading to the formation of a small cherry. This process can take up to 50 days, depending on the weather. Then, in the stage II the fruit starts to growth until it reaches its final size; however, the seed is still tender. When the coffee bean reaches its complete development is when the stage III starts, as a result the seed acquire its characteristic solid consistency and gains weight. In the last stage, the fruit development is complete, thus the plant starts to focus on the maturation of the cherry. In this stage the cherry changes its tonality from green to yellow or red-wine color. At this point the fruit is ready for harvesting (Medina-Meléndez, J. A., et al, 2016).

Focusing on the coffee cycle, the longest and most crucial stage is the fruit maturation process (**Figure 8**) because it will determine the quality and value of the coffee that will be produced. This stage has been changing nowadays, due to the warmer temperatures. Also, high humidity levels are essential in order to achieve a correct development of the fruit. Thus, since 2006, the floral cycle has been shifted and nowadays it is possible to observed mature fruit in August, where normally they should appear until October. This shows how the changes in the environment has been affecting the normal coffee cycle production (**Graph 3 and 4**). At the same time, the abnormal pattern of precipitation started in the same period where the first abnormal CRL outbreak was found (from 2008 to 2013) (**Graph 3**). A possible explanation could be that during the dispersion and germination

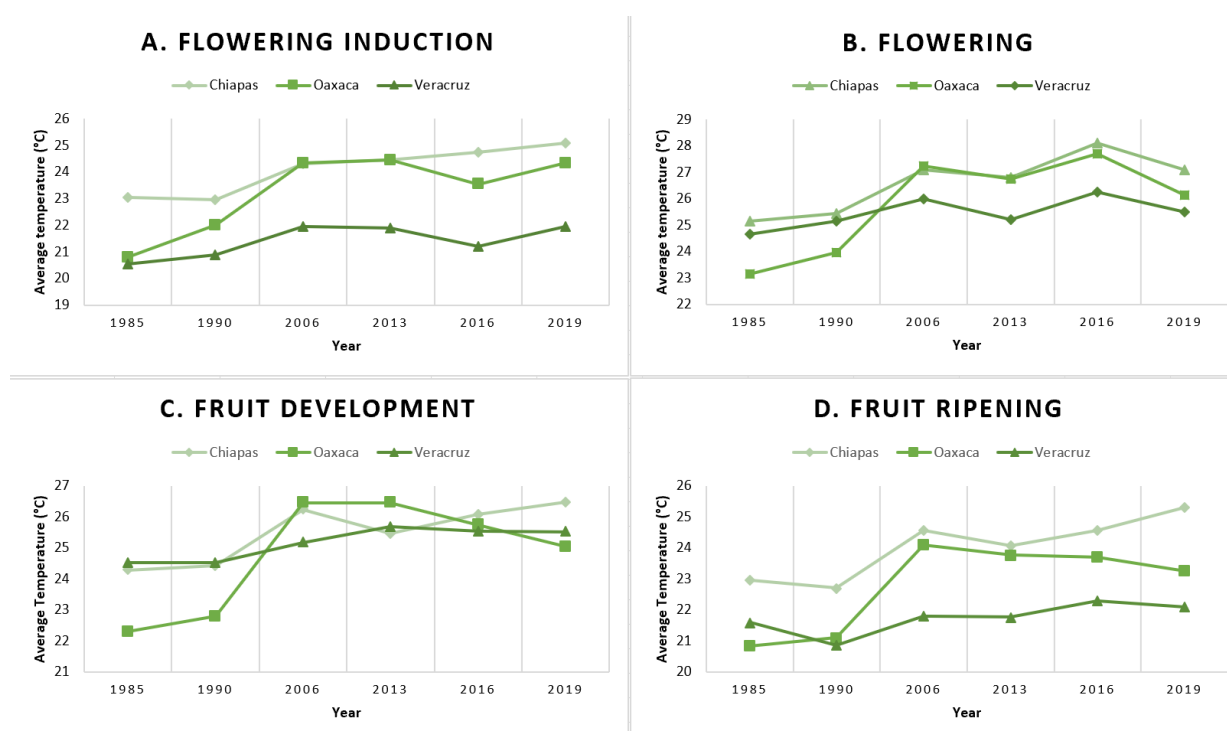
processes of HV, free water is needed. Therefore, CLR develops more easily during periods of continuous rainfall than in dry (Gichuru et al., 2012).



Graph 3. Precipitation dynamics in Chiapas, Oaxaca, and Veracruz between 1985 and 2019. It is observed highly variable patterns in precipitation. The plotted data was the corresponding to the months for each phase of the *Coffea arabica* cycle. For instance, (A) Induction to flowering, in the months of February and March. (B) Flowering in the months of April and May. (C) Fruit development from June to September and (D) Fruit ripening in the months of October to December. Data retrieved from the National Meteorological Service of Mexico (SMN).

Pests and diseases, especially CLR and the drill, will be decisive in the disappearance of the crop in the lower areas (Pérez and Villafuerte, 2018). Currently, the average temperature for these months has remained above 25°C being adequate for the development of HV and ceasing to be optimal for *Coffee arabica* in each stage of their production cycle (**Graph 4**). As consequence, coffee growers have been forced to move their crops at higher altitudes to keep their cultivars free of CLR and provide the *Coffee arabica* varieties with more suitable growth conditions (Talhinhas et al., 2017). However, the change in height interferes with the quality of the fruits, in addition to giving different nuances to coffee seeds. For instance, at altitudes below 1219 masl, a coffee with a light body and acidity,

soft aroma and regular fineness predominates. From this altitude to 1463 masl a pronounced brown coffee is produced with light acidity, great aroma, and regular fineness. As the latitude increase, full-body coffee prevails with light acidity, fragrant aroma, and low finesse. Due to this crucial attribution, despite the economic conditions of coffee growers, they cannot move their crops (Pérez and Villafuerte, 2018). Other important aspect for Mexican communities is the emotional attachment and the tradition behind coffee cultivation. This situation ends up by forcing them to do other type of economic activity as immigration, to maintain their heritage (Feng et al., 2010).



Graph 4. Temperature dynamics in Chiapas, Oaxaca and Veracruz between 1985 and 2019. It is observed highly variable patterns in temperature. The plotted data was the corresponding to the months for each phase of the *Coffea arabica* cycle. For instance, (A) Induction to flowering, in the months of February and March. (B) Flowering in the months of April and May. (C) Fruit development from June to September and (D) Fruit ripening in the months of October to December. Data retrieved from the National Meteorological Service of Mexico (SMN).

A mass migration across international borders may be the result if we continue leaving aside traditional crops and beating on monocultures. Hence, it will end in the loss of producing soils, due to erosion or drastic natural phenomena. On this

regard, according with Feng, S. *et al*, by 2080 is estimated that 2% to 10% of Mexican population (1.4 to 6.7 million) between 15 – 65 years could migrate due to the decline in agricultural productivity (Libert *et al.*, 2019).

The maturation process can take 32 weeks approximately. From the pollinized flower to the mature coffee cherry, the fruit passed for 4 major stages. The stage I takes place once the flower is opened and ready to be pollinized. When this occurs then the flower starts a slow transformation, leading to the formation of a small cherry. This process can take up to 50 days, depending on the weather. Then, in the stage II the fruit starts to growth until it reaches its final size; however, the seed is still tender. When the coffee bean reaches its complete development is when the stage III starts, as a result the seed acquire its characteristic solid consistency and gains weight. In the last stage, the fruit development is complete, thus the plant starts to focus in the maturation of the cherry. In this stage the cherry changes its tonality from green to yellow or red-wine color. At this point the fruit is ready for harvesting (Ghini *et al.*, 2011).

Even though the CLR crisis of 2012 – 2013 was not exactly a product of climate change, it is the most realistic framework of how climate change can impact the pest development (Chain *et al.*, 2019) Even though the CLR crisis of 2012 – 2013 was not exactly a product of climate change, it is the most realistic framework of how climate change can impact the pest development (Bejan *et al.*, 2018; Chain *et al.*, 2019). It is not new that global warming will generate serious problems for future coffee cultivation, due to quality reduction and productive problems related to coffee physiology. At the same time, it shows the areas that need to be strengthened in the agriculture sector, such as research, training, as well as generation, transfer, and adoption of technology (Aust and Hoyningen, 1986; Zadoks and Schein, 1980; Libert and Trench, 2016). To sum up, without additional efforts to strengthen the current adaptive capacity of native cultivars, there are likely to be large economic losses throughout the coffee supply chain, as well as the disappearance of ecosystem services.

Chapter 04

Strategic approaches to support sustainable communities

The current world situation points out the need for implementing conservation programs for long-term preservation of remaining and endangered coffee cultivars as *Coffea arabica* varieties *Bourbon* and *Typica* in Mexico (ICO, 2019). Even though, exists several places as Jaltenango, Chiapas, that have been producing coffee based on a sustainable scheme and without the use of chemicals, due to the increment in CLR, this approach is changing rapidly (SENASICA.,2016).

On the other hand, the demand of coffee is increasing as the population grows to the point of being one of the principal economical activities to 55 countries, generating an income only surpassed by tourism (ICO., 2020). In fact, according to the International Coffee Organization, in 2015, the world coffee consumption was 152.1 million bags (ICO., 2016). As result, from harvesting to consumption, more than two million tons of several residues are produced annually (Korekar G., et al, 2019) and according to the World Water Assessment Programme, around 80% of the total volume of wastewater generated is discharged without prior treatment (Quadra RG., et al, 2019). The whole process of coffee production generates at least five types of residues rich in caffeine, tannins, and polyphenols, which makes them very harmful for the living organisms (Korekar G., et al, 2019).

These practices have contributed to the increase of caffeine, and now it is catalogue as an emerging pollutant. This is why coffee producers use those residues as fertilizer, livestock feed, compost etc. to diminish the volume of residues. However, the difference between the waste that is generated and that which is used is still abysmal in coffee-producing areas (Banu., 2020).

Fortunately, global environmental awareness is increasing. Thus, strict legislation in recent years have focused research towards an eco-friendly control of pests and agroforestry as possible strategies to control CLR. On the other hand, the use of coffee waste as substrates for sustainable biorefinery can work as a new alternative to generate an income for sustainable coffee-growing communities in order to maintain their practices (Limousy L., et al, 2017; Banu., 2020).

4.1 Trending strategies for *Hemileia vastatrix* efficient control

It is important first to understand how HV interacts with the coffee plant. The devil is in the details and still so little is understood about its biology and role in nature.

Under this line of thoughts, the capacity of a pathogen to cause an epidemic will be depend on two factors: tolerance and compensation. Tolerance can be defined as an acquired condition developed by the survival to stress environments and it is hereditary. On the other hand, compensation is when the disease is developed despite the unfavorable weather conditions or during biological weakness, rather from the pathogen than by the host, but both cases can be presented (Zadoks and Schein, 1980). The fuse of both factors can be defined as coevolution, and this allows an optimal growth in concurrence with their hosts (Gautam et al., 2013). This has been the main phenomena acquired by HV during the decades, and both biotic and abiotic factors, including the human intervention, has played a crucial role in the current early development and increased aggressiveness of HV, as well as their incredible adaptive capacity (**Figure 9**) (Vidigal et al., 2019). However, despite of the adaptive characteristics of HV, the understanding of this pathosystem can also help in the control of CLR. By knowing the crucial factors, and by understanding the defense mechanisms and environmental responses to the CRL epidemic, focalized strategies can be developed to provide organic coffee crops with new options to face and survive the current crisis arising.

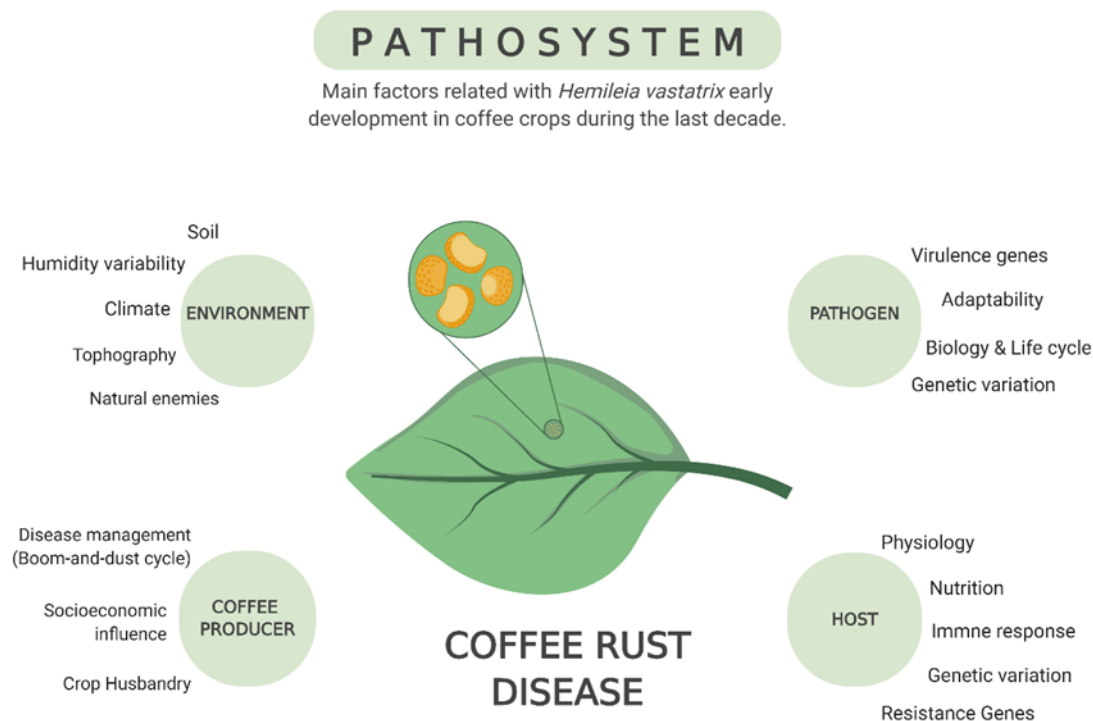


Figure 9. Factors involved in the pathosystem of *Hemileia vastatrix* and its early development based on the disease tetrahedron developed by Zadoks J. C. & Schein R. D. in 1980.

Currently, the list of functional fungicides has been drastically reduced due to environmental and health concerns. Hence, in recent years, this research field has increased for the development of novel and effective solutions for disease control. In the specific case of CLR, combination of salts has been developed with the aim to intensify their fungicidal properties. An example is the work of Vidigal, A. E. C. *et al*, where they developed nickel complexes with phosphines, and N-R-sulfonyl dithiocarbamates, containing triphenylphosphine ligands to inhibit the spore germination of HV. The composed denominated 2F was the most efficient with a 76% of inhibition (Carré *et al.*, 2014). On the other hand, potassium (PS) and calcium (CS) silicates, has been tested on the coffee leaf surface due to its ability to form a protective physical barrier and by its osmotic effect against some pathogens (Lopes *et al.*, 2014; Pérez *et al.*, 2019). In the first case, PS solutions

(20 g/L) were sprayed on coffee leaves infected showing a significant decrease in the progression of HV. As consequence, CRL only reached 32% of contamination after 36 days of the infection (Lopes et al., 2014).

In the case of CS, it was mixed with triadimenol and added on the coffee leaf surface in different concentrations. Plants treated only with CS had 94-97% of CLR incidence. In contrast, plants treated with CS and triadimenol did not exceed 6-16% incidence (Pérez et al., 2019).

Moreover, fertilizers may be playing an important role in CLR development as well. A study focused on the effect of nitrogen and potassium fertilizers shows that CLR severity increased 16.48% with a dose of 7 mmol N L⁻¹ and 11 mmol K L⁻¹ (Pérez et al., 2020). In contrast, other micronutrients as boron (B), manganese (Mn) and zinc (Zn) have showed reduction in severity of CLR (15.1%, 52.3% and 78.0%, respectively) at doses of 4.00 and 0.25 mg L⁻¹ (Osorio et al., 2018).

Other interesting approach is the evaluation of essential oils recovered from plants and spices commonly used in Mexico as possible control. Recently studies have revealed that essential oils promote cellular disorganization and cytoplasmic vacuolization on HV spores, inhibiting its germination (Pereira et al., 2012; Osorio et al., 2018). Oils recovered from cinnamon, citronella, lemongrass, clove, tea tree, thyme, neem, and eucalyptus were evaluated *in vitro* on HV uredospores and all the essential oils were able to inhibit the spore germination. Notwithstanding, once distributed on greenhouse coffee plantations, the most effective were thyme, clove, and citronella in a concentration of 1000 µL/L for each one (Cerna et al., 2019). In Mexico, these extracts have been proved in coffee varieties as *Typica y Caturra* as alternative to control HV severity (Zambolim, 2015). On the other hand, formulations based on elicitor molecules, such as BION (acibenzolar-5-methyl), Probenazole (ORYZEMATE) or beta-aminobutyric acid has demonstrated an increase in the incubation and latency periods of HV, leading to inactivation of the disease mechanism (Cepeda et al., 2014). Despite of the research required to be established as formal fungicides, either salts or essential oils, has shown to be promising environmental-friendly controls against CLR.

Myths and realities about shade crops and *Hemileia vastatrix* early development

In coffee crops in southern Mexico, levels of diversity have been found that are equal or even higher than those of natural forests. Some trees are planted with the expectation of fulfilling the shadow function for the benefit of principal coffee crops; others are vestiges of forests and fulfill the same function. This cultivation method influences beneficial organisms and natural pest management is also observed. One clear example is the Ant population (*Azteca instabilis*) that raise due to the tree abundance. This insect is considered as one promising biological agent for the control of CLR; however, its role as a biological control will be explained forward (Chain et al., 2019). Other important advantage is the increase in fruit quality, due to the extensive variety of pollinators attracted by the trees, such as spiders, butterflies, reptiles and specially birds (Mendoza et al., 2018). Also, in the canopy (formed by the treetops), on the trunks and the ground epiphytic plants, herbs and shrubs are established, and small mammals such as bats, rodents, possums, and foxes find refuge. In contrast, if coffee plantations are only surrounded by agricultural areas or fragmented environments, the diversity and abundance of these species decrease considerably (Gautam et al., 2013).

In Mexico, shaded coffee plantations are agroforestry systems that preserve the structure and composition of native forests, offering a place that allows the proliferation of a great diversity of flora and fauna. This, due to their altitude and climate conditions that coincide with the forests of the mountainous areas. In specific, rainforests (high, medium, and low height), pine and oak forests, and the mesophilic mountain forests, which are part of the ecosystems with the greatest biodiversity in Mexico (Pérez, E. and Villafuerte, D., 2018). At the same time, the Coffee plantations themselves can also contribute to mitigating climate change, since they trap and maintain carbon in their roots, in the accumulation of leaf litter, in the soil and tree trunks (Janissen and Huynh., 2018).

However, besides the advantages of traditional shaded cultivars, monocultures are increasing, mainly due to the ephemeral high productivity that this scheme provides at the beginning. According to Mendoza *et al.* to supply the demand for

land required for the agricultural sector (in specific monocultures), under non-sustainable schemes, 83% of deforestation in the country has taken place in thickets, temperate forests, evergreen forests and dry tropical forests, being the last two the most vulnerable (Villarreal, 2018). But why despite the disadvantages, non-sustainable practices are still the most applied? The reality is that smallholders depend entirely on the production of their land as primary source of income (Verburg et al., 2019), what makes them the most vulnerable in the market sector. Thus, adaptation must be implemented, but how still remains unclear. The biggest barriers are mainly, the lack of financial resources, access to knowledge networks and training material, and organizational support (Gomes et al., 2020). Thereby, some scientists and conservation organizations have promoted the certification “*Café amigable con las aves*”, whose productive practices favor birds: having reduced plot sizes, maintaining the composition and structure of forest, and having organic production. Also, allows producers to explore sustainable markets with added value, where consumers find a way to support the conservation of birds, both resident and migratory. This certification has been implemented in Oaxaca, Veracruz, and Chiapas (particularly, Sierra Madre de Chiapas and the Tacaná Volcano Biosphere Reserve) (Janissen and Huynh, 2018).

Table 3. Effects of the tree shade in reproductive routes of *Hemileia vastatrix*.

Action Route	HV Affected Stage	Potential shade effect
Rain	Dispersion	The shadow intercepts the raindrops. In light rains, water does not reach the coffee plants thus, no fungus dispersion is given.
Wind	Dispersion	The shade intercepts wind and reduces dry dispersion.
Dew	Germination and Leaf Penetration	Due to the shades, there is no dew in light rains, the only source of free water during the dry season.
Radiation	Leaf Penetration	The germination is favored with radiation reduction due to the shadow of the trees. Nonetheless, this also reduces leaf responsiveness to fungus inlet.
Fruitful Load	Leaf Penetration and Colonization	Tree shades help to maintain in equilibrium the coffee fruit production. Therefore, there is not overproduction nor low production, which allows maintaining the physiological

		resistance to the fungus.
Biocontrol	Sporulation	Tree shadows favor the growth of HV myco-parasites (<i>Lecanicillium lecanii</i>).

Even though, there is not direct consensus between the advantages and disadvantages of the shadow sowing method over the control of HV, some clues have been found related to shade tree effects (**Table 3**). It has been proven that the shadow method maintains almost the same productivity as coffee sown in the sun and help maintain up to 75% of the land used still suitable for coffee crops, as long as the shade supplied does not exceed 50% (CONABIO, 2015; Arneson, 2001). For instance, studies have established that *Coffee arabica* can maintain the photosynthetic requirement with less light than other species, which means that requires a lower solar incidence to maintain coffee productivity (Gichuru et al., 2012). Finally, high levels of sucrose, chlorogenic acid and trigonelline in sun-grown grains leads to incomplete maturation, which can result in crop weakness against any disease (CONABIO, 2015). In contrast, under shaded systems the maturation is delayed for up to a month, allowing a better adaptation and nutrition uptake from the plants to maintain their immune systems alerts. The shade provided by trees generally reduces the physiological stress of the plant which allows it to increase resistance to pests and opportunistic diseases as CLR. In addition, facilitates air circulation and rapid drying of the canopy, thus, avoiding HV sporulation and germination.

Besides shade, it is fundamental to keep in mind that trees also provide other services such as nutrient recycling and microclimate regulation. Shaded cultivars induce nutrient recycling, and the concentration of P and N in the soil, at the same time they worked as buffers to maintain the pH balanced. Furthermore, they hinder the transfer of fungal diseases by serving as windbreaks, slowing the spread of spores. Also, prevent erosion, increase water retention, and soil moisture content without competing with the coffee cultivars due to the structural difference, size and vertical arrangement of the roots (Berndt, R., 2012). On the other hand, trees help in reduce weed development (CONABIO, 2015). This is an important advantage, because coffee plants are very sensitive to weed competition, so its control

maintain the plant vigor, thereby increasing their capability to resist CLR outbreaks (Miller, 2001; Gomes et al., 2020).

Fundamental role of native biodiversity in *Hemileia vastatrix* management

To protect the highly susceptible coffee varieties, since 1980 the use of native soil organisms and their secondary metabolisms have been studied mainly in Colombia, Brazil and India as a possible alternative to control HV proliferation, instead of chemical fungicides which are prohibited in organic cultivations. Organisms as *Bacillus thuringiensis*, *Pseudomonas fluorescens*, *P. aureofaciens*, *P. alcaligenis*, *P. putida*, *Simplicillium* sp., *Fusarium* sp. and *Lecanicillium lecanii* has shown promising results as HV antagonists (Cepeda et al., 2014; Gómez et al., 2018; Cacefo et al., 2016).

Hence, based on the natural antagonism, *L. lecanii* is an ascomycete fungus, primarily entomopathogen of *Coccus viridis*, which is an insect that works together with the arboreal nesting ant *Azteca instabilis* (mutualistic relationship). This beneficial agreement between those organisms, allows *L. lecanii* to reach the HV spores located in coffee leaves to parasite them, reducing its viability and aggressiveness. This type of complex interactions demonstrates the importance of native biota, as control agents (Wintgens, 2004; Cacefo et al., 2016). However, to exploit *L. lecanii* maximal potential, demanding conditions are required, which decreases its field reproducibility and generates high production costs (Gómez et al., 2018; Cacefo et al., 2016).

On the other hand, as preventive control, several strains of *Bacillus* sp. have been suggested as preventive treatment for coffee crops as *B. subtilis*, which has acted effectively in control of HV, but with less efficiency than the commercial products like azoxystrobin (Diniz et al., 2012). In the case of *B. thuringiensis* isolated strains, studies have shown inhibition of the uredospores germination in a range from 77 to 97% compared with no treated plants. The efficiency range was attained by inoculating commercial strains (*B. israelensis* and *B. kurstaki*) before any contact with HV uredospores. Thus, proven the same effectiveness that commercial products as *M-One*® (Vidigal et al., 2019).

Moreover, *Beauveria bassiana*, has shown promising components to manage CRL rapid proliferation. In a laboratory scale, toxins extracted from *B. bassiana* (the secondary metabolisms Beauvericin and Basianine) were proven in coffee crops (*Caturra* variety), helping the plants to develop induce resistance against HV. This induce resistance could be the result of the increment of Lipoxigenase, β -Glucanase and Chitinase content in the coffee plants (Cepeda et al., 2014).

The latest discovery regarding potential biocontrol, using the native organisms of coffee crops, was found in Puerto Rico, a gastropod community able to consume HV uredospores produced once the CLR infection reaches the mature stage. This is considered as a prominent discovery because invasive snails were catalogue as herbivores, until now. The study leads by researcher from the Michigan University found that *B. similis* was able to only to consume approximately 30% of the HV uredospores produced in a leaf, 24 hours later, but also its mycoparasite *L. lecanii* without affecting the leaves (Herrera et al., 2019). Therefore, bringing the opportunity to explore new management techniques based on the adaptation and evolution of the native organisms found in the Coffee agrosystems. Other interesting perspective is endomycorrhic populations present in coffee varieties with and without signs of CLR. On this regard, in Ixhuatlán del café, Veracruz were identified 37 species that correspond to 14 genera of endomycorrhizal, being the most common *Acaulospora* and *Rhizophagus* (the last one most frequent in plants without signs of CLR). The abundance of mycorrhiza in coffee cultivars demonstrate a significant capacity to tolerate better the biotic and abiotic stress, including CLR (Echeverría et al., 2019).

Soil and Plant Nutrition for crop reinforcement

For any type of cultivar, the quality and quantity of nutrients (which can be a wide variety of organic and inorganic compounds) is an important factor to ensure a high yield and resistant crops against pests and unfavorable environmental conditions. Their availability is determined by the physical, chemical, and biological characteristics of the soil (Baba et al., 2020), and its principal role is to allow the complex interactions between plants, animals, microorganisms, and fungi. The soil

works as a versatile matrix, that contains three phases (liquid, solid and gaseous) and each phase interacts with different types of nutrients in response to maintain balance for optimal plant development (Pandey, 2015; Baba, 2020). The solid phase function as a pool of organic compounds, which are sources of (nitrogen) N, phosphorus (P) and sulfur (S), and inorganic minerals (commonly negatively charged tetrahedral particles) as (potassium) K, calcium (Ca), magnesium (Mg) and iron (Fe). On the other hand, the liquid phase contains dissolved ions that are transported towards the plant roots, contributing to the nutrient absorption. Inside the soil structure, spaces are formed and occupied by gases like carbon dioxide (CO₂), N and oxygen (O₂) (Bala, et al., 2018).

The base compounds for plant survival are Carbon, Hydrogen, and Oxygen; notwithstanding, mineral nutrition is also crucial to maintain plant homeostasis and vital functions, as proper growth, tissue health and solid structure, reproduction, and a well-developed immune system. Due to its importance, mineral nutrition can determine the resistance against several diseases, including CLR (Spann and Schumann, 2010). The nutritional status has a remarkable impact on coffee crops to overcome the CLR crisis, due to the energy required for fruit development, and during the absence of nutrients it has been observed an increment in the severity of HV, even in the subsequent years. In specific, a mineral imbalance weakens the cell wall of the leaves, increases the concentration of sugars and amino acids in both leaf and stem tissues, which ends up creating a more favorable environment for HV development (Shrivastava and Kumar, 2015).

Tissue calcium content could be strongly correlated to fungal resistance due to its capacity to inhibit the fungal enzymes, which dissolve the component that bonds adjacent cells in leaves (known as lamella) (Spann and Schumann, 2010; Bala et al., 2018). On the other hand, Boron addition can help to control HV by enhancing defense compounds able to hinder fungal attacks. As the plant agents, the accumulation of Silicon in the leaf surface leads to the formation of mechanical barriers that prevents fungal penetration. The integration of this mineral can be implemented for younger crops (Miller, 2001; Pandey, 2015). Other important nutrient is Copper highly used as a fungicide, nevertheless the amount needed to

have effects on the fungus is well above the plant's nutritional needs. This can affect the innate immune system (Shrivastava and Kumar, 2015).

Even though, mineral nutrition can greatly favor the resistance of the plant against CLR, it is very important to consider both the amount to affect the fungus and the nutritional requirements of the host (**Table 4**). An excess of minerals can lead to the weakening of the plant and thereby increase the susceptibility to diseases (Miller, 2001; Munns and Tester, 2008; Bala et al., 2018).

Table 4. Mineral nutrition: Principal elements and average percentage in shoot dry matter required for adequate growth of coffee plants (Miller, 2001; Munns and Tester, 2008; Bala et al., 2018).

Element	Required form for plant nutrition	Concentration in dry tissue (%)
Molybdenum	MoO_4^-	0.00001
Nickel	Ni^{2+}	0.00001
Copper	Cu^+, Cu^{2+}	0.0006
Zinc	Zn^{2+}	0.002
Manganese	Mn^{2+}	0.005
Iron	Fe^{3+}, Fe^{2+}	0.01
Boron	H_3BO_3	0.002
Chlorine	Cl^-	0.01
Sulfur	SO_4^{2-}	0.1
Phosphorus	$HPO_4^{2-}, H_2PO_4^-$	0.2
Magnesium	Mg^{2+}	0.2
Calcium	Ca^{2+}	0.5
Potassium	K^+	1.0
Nitrogen	NO_3^-, NH_4^+	1.5
Oxygen	O_2, H_2O	45.0
Carbon	CO_2	45.0
Hydrogen	H_2O	6.0

Breeding and Genetics

Breeding techniques are considered the best strategy for disease management that combine both economic and environmental benefits (Herrera and Lambot,

2017). They have been widely applied around the world, and with the CLR outbreak many institutions and programs were created with the aim to characterize rust and the *Coffea sp.* genome to coordinate strategies focused on new coffee varieties development (Wintgens, 2004). So far, only nine genes involved in the resistance of *Coffea sp.* are known (from SH1 to SH9), which correspond to the virulence factors in HV (from v1 to v9). The non-race-specific resistance genes (SH genes) are used to determine *Coffea sp.* resistance groups, while virulence factors determine physiological races of HV (until now 50 are known) (Avelino and Rivas, 2013; Flor, 1954; Silva et al., 2002). Hence, the perfect example of breeding results are the HdT offspring, which have obtained their resistance due to the combination of genes derived from Robusta (SH6, SH7, SH8, SH9), Arabica (SH1, SH2, SH4, and SH5), and Liberica (SH3) (Chain, 2019).(Alvarado et al., 2009). However, this resistance is being lost and HdT-derived varieties are getting infected by HV, leading to the investigation of new resistance sources (Alvarado et al., 2009). Thus, crosses between *Robusta* and *Arabica* are being performed, as well as genetic analysis with the aim to find promising resistance markers (Ovalle et al., 2015; Chain et al., 2019; Alvarado et al., 2009). Besides HdT, another promising variety, recently developed in Central America is the hybrid of first-generation (F1). These hybrids are well-adapted to a diverse range of environments, especially shaded cultivars, without reducing their productivity (30-60% above the traditional cultivars production). This characteristic makes them the perfect candidate for ecologically intensive agriculture schemes. Nonetheless, their need for vegetative systems increases considerably the costs of coffee plantlets (Herrera et al., 2019). Therefore, approaches as seed propagation or *vitro* propagation combined with horticulture are being studied (ICO., 2019).

Exists several organizations focused on addressing the potential decline in coffee supply predicted for the future years. As an example, *Breeding for the future* is a program lead by the World Coffee Research with the aim to develop new varieties able to tolerate changing weather patterns, increased temperatures, disease prevalence, without excluding the quality of the cup (Diniz et al., 2012). On the other hand, since 2010 the Food and Agriculture Organization of the United

Nations (aka as FAO) has encourage several programs in Central America directed to technological development for data compilation to evaluate the socio-economic impact of CLR in real-time that will help to generate early warning systems and study the most suitable regions to cultivate resistant varieties (Cepeda et al., 2014).

Finally, there are still many wild *Coffea* species that have not been discovered. This brings us the opportunity to discover new genes related to resistance, organoleptic properties, or particular characteristics, which could be crucial for *Coffea* sp. improvement. The species *Coffea charrieriana* and *Coffea anthonyi*, both discovered in Central Africa, are the most recent examples. The former has the unique quality of being the first naturally caffeine-free species discovered in that area. The second one is a small-leaved type that possesses a self-compatible mechanism of reproduction, which is highly rare within the genus *Coffea* (ICO,2019).

As future outcome for coffee breeders in the coming decades, the priority must be the implementation of DNA-based tools (as selection based on molecular marker) to speed up the development of new Arabica varieties with a strong tolerance to biotic and abiotic stresses, such as CLR and higher temperatures. This must be achieved without losing sight of the outstanding attributes of *Arabica* varieties, as their sensory qualities, and considering the environmental importance of the endangered and ancient coffee species (Ovalle et al., 2015).

4.2 The use of coffee husk as a bio-adsorbent to tackle caffeine contamination.

At the present time, caffeine is considered the most popular psychostimulant and analeptic, constituting 75% of the non-alcoholic beverages market (Delgado S., et al, 2019). Besides food products and drinks, it is also abundant in prescription drugs (such as analgesics, antihistamines, diet pills, cold remedies, and stimulants of psychophysical activity) even though it is not an essential nutrient (Anastopoulos I., et al, 2020).

Caffeine (1,3,7-trimethyl-3,7-dihydro-1H-purin-2,6-dion) is a methylxanthine alkaloid with chemical formula $C_8H_{10}N_4O_2$ that acts as a stimulant to the central nervous system in humans to maintain alertness. The main routes of caffeine wide distribution have been coffee processing and human excretion, despite the high degradation rate (Li S., et al, 2020). Regarding human consumption, 3% of the caffeine is excreted as urine (Anastopoulos, I., & Pashalidis, I., 2019). Even though this percentage seems harmless, we have to consider that over 2.25 billion cups of coffee are consumed in the world every day (International Coffee Organization, 2015). This triggers the amount of caffeine that is released into the environment. Even though in the past was catalogued as a safe compound; nonetheless, its high concentrations can change the physicochemical characteristics of soil and water bodies, leading to the disruption of physiological processes, and affecting the reproductive function of living organisms at macro and microscopic level, including humans (Quadra RG., et al, 2019; Anastopoulos I., et al, 2020). This high demand has transformed caffeine into a well-known marker of domestic wastewater contamination, classifying it as an emerging pollutant (Czech, B., & Tyszczuk-Rotko, K., 2019). Caffeine is found naturally in beans, leaves, and fruits in about 60 plant species as coffee, cacao, or tea; However, *Coffea arabica* and *Coffea canephora*, are the plants with the highest content. Nowadays, these crops are cultivated in at least 80 countries across the globe and entangles huge businesses worldwide (Korekar G., et al, 2019).

Becoming aware of caffeine impact: Human metabolism, coffee processing and ecotoxic effects.

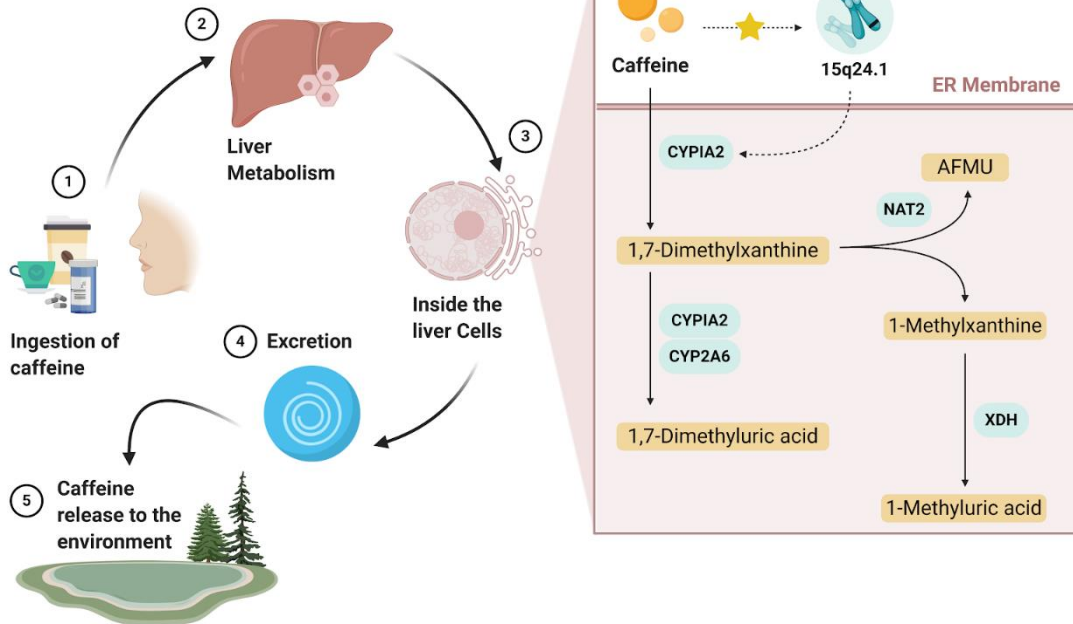
The kinetic metabolism of caffeine in coffee and tea plants is derived from purine nucleotides, mediated by xanthopsin methyltransferase and theobromine synthase followed by caffeine synthase. In humans, caffeine is absorbed by the gastrointestinal tract, and metabolized in the liver. Lately, close to 3% of the caffeine intake is delivered to the kidneys, but here remains unchanged and is excreted as urine (Qian, J., et al, 2020; Anastopoulos I., et al, 2020). However, this range of time may be prolonged in patients with hepatic diseases, during

pregnancy, for infants and neonates, where it can take up to 100 hours to be complete digestive (Nehlig, A., et al, 2018; Qian, J., et al, 2020). In general, around 70% of the caffeine and its metabolites are recovered in urine, meanwhile, around 2-7% of the caffeine is excreted in feces over 48 hours (Nehlig, A., et al, 2018). Caffeine and its metabolites could undergo demethylation, oxidation, hydroxylation reactions, and the oxidation of caffeine is mediated by the gene *CYP1A2*, which encode to the hepatic protein cytochrome P450s (CYP) 1A2 and 2E1. The main metabolism route occurs in the liver, where the CYP1A2 carries a *N*-3-demethylation to paraxanthine known as 1,7-dimethylxanthine (70-80% of the caffeine). Approximately 7-8% of the caffeine undergoes 1-*N*-demethylation to theobromine, 7-8% has a 7-*N*-demethylation to theophylline, and, the 15% remaining go through a C-8 hydroxylation to form 1,3,7-trimethyluric acid (Qian, J., et al, 2020). Besides paraxanthine, the major metabolites of caffeine found in urine are 1-methylxanthine, 1-methyluric acid, 5-acetylamino-6-formylamino-3-methyluracil, and 1,7-dimethyluric acid, which are formed during a secondary metabolism of paraxanthine by cytochrome P450 CYP1A2, CYP2A6, *N*-acetyltransferase 2, and xanthine dehydrogenase (also known as xanthine oxidase). **(Figure 10.1)** Many of these metabolites could affect the metabolism of plants and animals and could become toxic for the environment (Rigueto et al., 2020).

On the other hand, the other route of importance for caffeine release is coffee processing. During harvesting, the first residues found are pulp and husk of the fruit and leaves of the plant (Quadra RG., et al, 2019). Later on, to dehulled the fruit exists three possible pathways: dry, semi wet or the wet method (the most popular), during this process also fermented water will be generated and discharged to the environment (Li S., et al, 2020). Moving forward, once the grain reaches a humidity of 11%, then the silver skin is removed, as well as the defective beans. Finally, after roast and ground the coffee beans, the coffee is ready to be prepared and it is here when the last by-product appeared: the spent ground coffee **(Figure 10.2)** (Delgado S., et al, 2019).

Main Routes of Contamination

1. Pathway of caffeine consumption



2. Pathway of Coffee processing and by-products generated

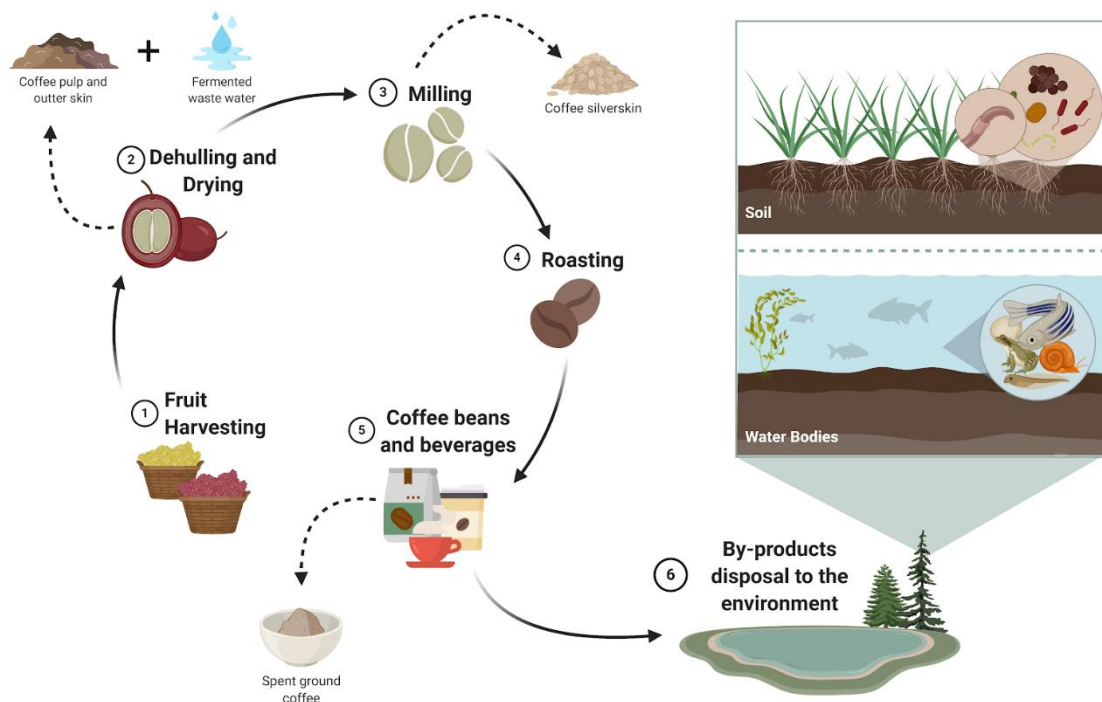


Figure 10. Main routes of caffeine contamination in soil and water bodies.

The figure 6.1 shows the route of caffeine consumption, where 3% of the total caffeine metabolized by the human body is released to the environment. In the figure 6.2, it is observed a brief resumé of coffee processing

and their by-products. The wastes generated reach both landfills and water bodies, this includes rivers, lakes, underground aquifers, seas, and the ground. As a result, the high levels of caffeine affect the native flora and fauna, due to the acidification of the medium, generating fertility problems and congenital anomalies. Created with Biorender.

The release of pharmaceutically active compounds, such as caffeine, generated by human and industrial activities has become an emerging contaminant in the natural environment. This distribution includes humans and animals' excretion, inappropriate disposal of products, manufacturing plant wastes, intensive crops, landfill leachates, storm drainage in rural and urban areas, and outflows from wastewater treatment plants, among others have a great impact on the natural environment, including marine and freshwater ecosystems (Li, S., et al, 2020). Higher residual caffeine concentrations are often found in sampling locations closer to densely populated areas, according to caffeine sources and population consumption. However, areas isolated from human influence are not exempt such as the Antarctic (Czech B., & Tyszczuk-Rotko K., 2019), which shows that there is an increasing serious environmental issue.

Residual caffeine concentrations have been found in wastewater, treated wastewater, rivers, groundwater, drinking water, lakes, catchment areas, reservoirs, rainwater. This serious caffeine pollution is likely to be due to the collection of wastewater samples from industrial areas with multiple industrial sectors, such as food processing industries, breweries, and pharmaceutical industries, which apply caffeine as an ingredient into their products. (Li, S., et al, 2020)

Caffeine has been frequently detected in agricultural produce and there has been little attention to its metabolite products in vegetables. Many pharmaceuticals have been reported to be metabolized in plants, and their intermediate or end products could still contain bioactive functions. For example, caffeine and its metabolites in aqueous solution and lettuce were identified and underwent demethylation reactions. The risk assessment of pharmaceutical products, such as caffeine,

without considering their metabolites could underestimate the damage to the ecosystem and human health (Qian, J., et al, 2020).

The absence of chronic or acute toxicological effects does not exclude the possibility of other kinds of effects such as the presence of mutagenic effects, or effects in native organisms with longer life cycles under chronic exposure (such as fishes). These exposed organisms could be valuable indicators of possible toxic effects (Delgado S., et al, 2019).

Caffeine and its metabolites bioaccumulation should not be underestimated, and overdose exposure of caffeine to vulnerable pediatric and adolescent individuals would result in neurologic and cardiac toxicities, prenatal caffeine exposure in rats increased the progeny the susceptibility of metabolic disorders. Also, there is evidence that the death of some aquatic organisms might be led by long-term exposure to trace levels of caffeine (Anastopoulos I., & Pashalidis I., 2019). The evaluation of the presence and concentrations of the emerging pollutants with assessment of the potential risks, is essential to better understand the impacts of these pollutants as caffeine. It is necessary to have more research about the real effects on the ecosystem, including community analysis, the use of biomarkers, and bioassays with other test organisms (Quadra RG., et al, 2019).

Caffeine stripping based on a circular economy approach.

The high consumption of coffee has turned caffeine into an emerging pollutant and can reach soils and water through different routes. Although waste treatment plants provide a good percentage of compound removal, this efficiency varies in each treatment plant. Also, incorrect disposal of the residues from companies and smallholders contribute to this problem. Different strategies are used for caffeine removal, the most common are oxidative processes, biodegradation, separation by membranes, and adsorption (Rigueto et al., 2020).

In the case of oxidative processes, exists multiple oxidative treatments with hydroxyl radicals, sulfates, zinc oxides, titanium oxides, and cobalt derived

materials. This option shows a high efficiency regarding pollutants removal, and caffeine is not an exception (Lin et al., 2018; Li et al., 2020).

Other alternative is biodegradation by the use of microorganisms and enzymes, which are a fundamental part of wastewater treatment plants nowadays. In the case of caffeine removal, it represents an ecological, sustainable, and low-cost option (Win et al., 2019). In the membrane separation processes, the membranes act as physical barriers with variable selectivity based on the size of the pore and the compound to be separated. It also involves the flow rate, loads of the membrane with the molecule of interest, among other aspects (Kim et al., 2018).

Finally, we have the adsorption process, which is the separation of substances, immersed in liquids or gases by binding them to the outer or inner surface of an adsorbent. The sorption mechanisms can be physical, chemical, or a mixture of both (Crini et al., 2019). Among the emerging pollutants treated by adsorption, we can find Dyes, heavy metals, toxic industrial effluents, fertilizers, and pesticides, and in this specific field, bio-adsorption has emerged as a potential solution for the treatment of emerging pollutants. This, due to its simplicity, low production costs, and the wide range of materials that can be employed for this technique (Singh et al., 2020).

Bio-adsorbents

Bio-adsorbents are classified according to their origin as natural, biological, and derived from agro-industrial waste. Natural ones are those that are available in the environment as clay, siliceous, zeolite, among others. Biologicals are those made from biomass of bacteria, fungi, algae, and higher animals such as crustaceans and insects. Finally, there are agro-industrial wastes (Singh et al., 2020). The adsorbents derived from agricultural waste have been the most used in the removal of caffeine, in comparison with the commercial options (**Table 5**).

Table 5. Bio-adsorbents with a potential use for caffeine removal in aqueous media, and their adsorption capacity (Riedel, S.L., & Brigham, C., 2019; Anastopoulos., et al., 2020; N'diaye, A. D., & Kankou, M. S. A., 2020; Rigueto, C. V. T., et al., 2020; Keerthanan, S., et al, 2020).

Type of bio-adsorbents	Classification*	Maximum adsorption capacity (mg. g^{-1})
Tea Leaves	N	435 ^{b,d}
Raw grape stalk	N	89.194 ^e
Grape stalk modified by phosphoric	M	129.568 ^e
Activated carbon obtained from grape stalk	M	395 ^e
Native dead biomass of <i>Trichosporon</i> sp.	B	379 ^b
Dead biomass of <i>Trichosporon</i> sp. VITLN01 pretreated with Tween 80	M	766 ^b
Dead biomass of <i>Trichosporon</i> sp. VITLN01 pretreated with SDS ^a	M	471 ^b
Dead biomass of <i>Trichosporon</i> sp. VITLN01 pretreated with NaOH	M	448 ^{b,d}
Water hyacinth biochar obtained from pyrolysis	M	2.4488 ^{b,d}
Washed fique bagasse biochar pyrolyzed at 650, 750, and 850°C	AW	3.5162 ^b – 4.5263 ^f
Unwashed fique bagasse biochar pyrolyzed at 650, 750, and 850°C	AW	4.0288 ^b – 9.1296 ^f
Activated carbon from coconut waste	AW	171.23 ^b
Activated carbon fibers prepared from pineapple plant leaves	N	155.50 ^b
Oxidized carbon from <i>Luffa cylindrica</i>	N	59.88 ^{b,d}
Oxidized pine needles biochar	N	5.35 ^{b,d}
Powdered groundnut shell	M	4.21 ^b
Activated carbon from <i>Eragrostis plana</i> Nees leaves	AW	272.3 ^b
Charcoal from rice husk	AW	2.09 ^b
Charcoal from rice husk blended with corn cob	AW	8.04 ^b
Composite from coffee residues and chitosan	AW	8.66 ^c
Sepiolite from natural clay	N	48.7 ^b
Activated carbon	N	271 ^b
<i>Gliricidia sepium</i> biochar pyrolyzed at 300, 500, and 700 °C	N	16.26 ^d

a. SDS = Sodium dodecyl sulfate.

b. Values predicted by the Langmuir isotherm model.

c. Values predicted by the pseudo-second order kinetic model.

d. Values predicted by Freundlich isotherm model.

e. Values predicted by Sips isotherm model.

f. Values predicted by Redlich-Peterson isotherm model.

*Classification according to their treatment and origin, wherein N = natural; M = modified; B = biologic; and AW = agroindustrial waste.

Among natural bio-adsorbents the capability of modified green mud bentonite to adsorb up to 0.73 mmol / g of caffeine at 60 ° C was reported (Oliveira M. F., et al., 2019). Also, in other studies, with calcified bentonite at 500 ° C, a caffeine removal of 90% was achieved under optimized conditions and with a caffeine adsorption capacity on bentonite of 41.7 mg/g (Lenzi et al., 2020).

For the most part, agricultural wastes cannot be used directly as bio-adsorbents; they must undergo a series of chemical and physical transformations (Huang et al., 2020). To date, they are the most explored as mechanisms for the treatment of emerging pollutants and are the most widely reported for the removal of caffeine due to their high availability, low cost, completed adsorption efficiency, easy recovery, reuse capacity, and secondary products a once the treatment finished, they are usually not polluting (Huang et al., 2020), Pine tips with an adsorption capacity of 6.26 mg/g carbonized at 650 ° C (Anastopoulos et al., 2020), datestones of *Phoenix dactylifera* subjected to pyrolysis at 900 ° C with an adsorption capacity of 12.5 mg/g have used (Danish, M., 2020), activated carbon from bovine bone functionalized with MgAl and a double sheet of hydroxide groups (LDH), was used for the elimination of caffeine in water with an adsorption capacity of 26.3 mg/g (Dos Santos Lins et al., 2019). On the other hand, the agro-industrial residues mainly available such as the grape stalk have shown a high capacity as a bio-adsorbent, only the stalk without any treatment other than the modification of its size particle, reached an adsorption capacity of 89.2 mg / g, treated with phosphoric acid reached 129.6 mg/g and converted to activated carbon it a maximum adsorption capacity of 916.7 mg/g (Riedel, S. L., & Brigham, C., 2019). Another bio-adsorbent, this time derived from pineapple plant leaves and treated by slow pyrolysis in the presence of H₂SO₄, has shown good capacities to adsorb caffeine, reaching an adsorption capacity of 155.5 mg/g (Beltrame et al., 2018). Other types of bio-adsorbents derived from agro-industrial waste have been converted into activated carbon and used for this purpose with less promising results, such as fique bagasse (Correa-Navarro et al., 2019), dry biomass of *Gliricidia sepium*, or tea residues (Keerthan, Bhatnagar, et al., 2020).

A solution that fits the problem: Coffee husk as bio-absorbent for caffeine removal

The circular economy proposes optimal management of biological resources. In the same way, it has given rise to agro-industrial waste having a different purpose than composting. Actually, they are the support of different types of biomaterials with applications in industries cosmetic, food, medicinal and environmental. An outstanding example, coffee residues have emerged as a promising agro-industrial waste with multiple applications, including the treatment of contaminants, due to their abundance. According to the International Coffee Organization (ICO: www.ico.org), until august of 2020, 10.04 million bags of coffee have been exported, which is equivalent to 700,000 tons, for which 420,000 tons of waste generated in the production process, that is, only between mucilage of coffee (MC), the silverskin (SS) and coffee husk (CH), it is not considering that for every kg of coffee consumed 2 kg of spent ground coffee (SGC) produced.

The coffee husks (CH) obtained after peeling the cherries from it contain a high content of cellulose (24.5%), hemicellulose (29.7%), lignin (23.7%), and ash (6.2%) to which limited applications are like it's in fertilizers, livestock feed, compost, etc (Rajesh Banu et al., 2020). In recent years, it has sought to take advantage of this waste in such a way that it has been used to improve the mixture of biofilms of biocompatible PLAs, for the elaboration of micro and nano cellulose crystals and inhibitors of steel corrosion (Collazo-Bigliardi et al., 2019). One of the most promising applications of coffee husks is their use for the production of activated carbon by pyrolysis. Under this scheme, it found that activated carbon from coffee husks can adsorb contaminants such as malachite green dye with an adsorption capacity of 263 mg/g when treated with H₂SO₄ before carbonization, and this process carried out at a temperature of 200 ° C (Krishna Murthy et al., 2019), It can also adsorb methylene blue with a capacity of 415.8 mg/g when its carbonization occurs at 180°C (Tran et al., 2020). Heavy metals have been treated as adsorption efficiencies for the case of Cu 98%, Cd 85%, Zn 79% with the unscarred coffee husk but with a washing treatment, drying at 105 ° C and subsequent treatment. with formaldehyde, NaHCO₃, NaCO₃, and NaOH (Oliveira M.F., et al., 2019).

Also, when the coffee husk undergoes slow pyrolysis and as the temperature increases, a high number of cracks and pores are generated due to the expulsion of the volatile compounds contained in it, thus increasing its adsorption capacity. different substances like caffeine (Setter et al., 2020). The use of this dry shell or as activated carbon as a bio-adsorbent can be a matrix to treat caffeine in remote places where coffee and water sources are contaminated. It could be a solution economical, safe, and easily usable by people without many specializations in the subject.

4.3 Adding coffee by-products to the value chain as a sustainable pathway for energy production.

Different extraction methods are used to obtain the bioactive compounds; these methods we will not go approach in this review; we will only focus on the use of each of the residues generated throughout the processing, consumption, and final waste of coffee like a substrate for the generation of different types of energy.

As mentioned previously, along with the coffee production and consumption chain, four groups of waste generated that can have a high energy value or serve as a substrate for the generation of biomass (**Figure 11**). The different high-value products obtained from these residues depend mainly on the extraction method used, and the efficient use will depend on the understanding of each of these extractive processes (Janissen, B., & Huynh, T., 2018).

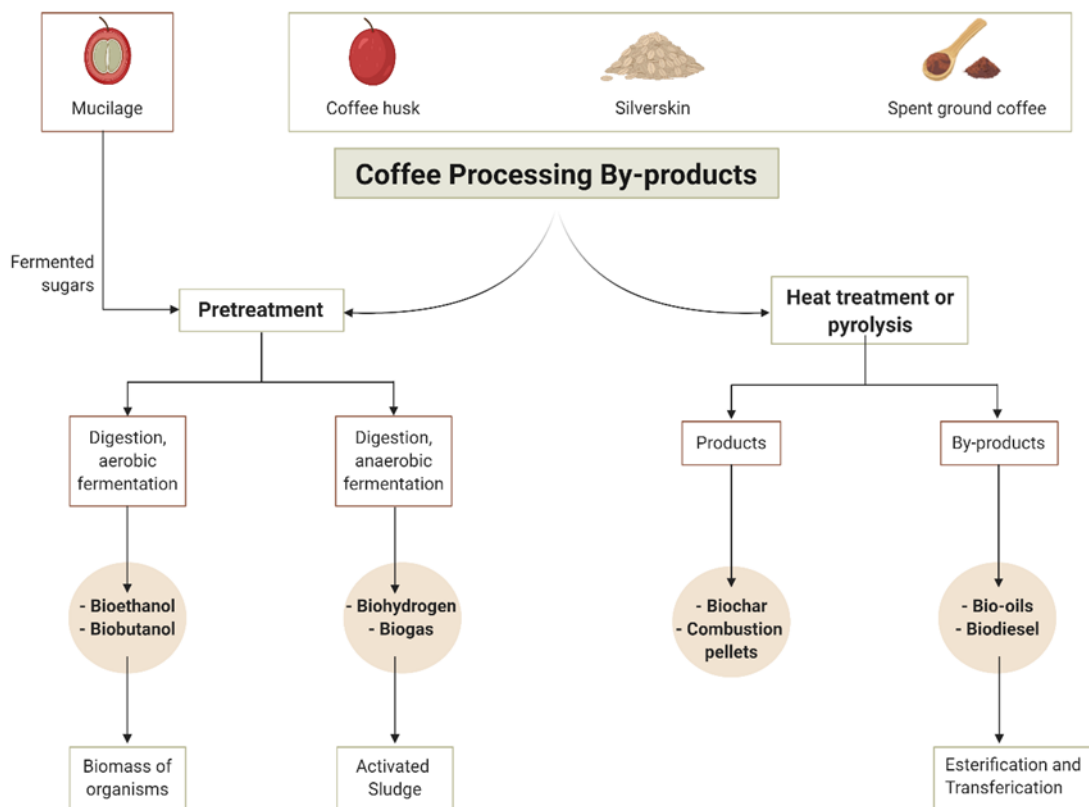


Figure 11. The harnessing process of coffee by-products to transform them into energy. All the by-products ended up in biodegradable wastes. In the case of the Bio-oils, like glycerol, and Biodiesel, as they are the final products, no waste is generated in this step. Regarding biorefinery, the coffee pulp is the only by-product that is directly used for digestion. It is not suitable for pyrolysis due to its water content.

Energy and biofuels produced from pyrolysis processes

In recent years, SGC, SS, and CH have been used to produce thermal combustion pellets or briquettes, which are dry, compacted materials that generate good energy in the form of heat after combustion. Another application of these three groups has been their use in direct pyrolysis to generate biocarbon. In both applications, the process by which the combustion kinetics passes can contribute to the generation of high-value products such as bio-oil and biodiesel. An analysis of the solid and liquid fraction of the slow pyrolysis of combustion pellets to The splitting of coffee husks (CH) showed that at higher temperatures, the bio-oil had a higher composition of phenolic groups and nitrogen compounds, when this temperature increases in the final stage, the quality of the bio-oil is improved, which can later be used as fuel, at lower temperatures it is it promoted the

formation of bio-hydrocarbons and acetic acid. Also the carbon performance decreases as the temperature increases (Setter, C., et al, 2020a; Setter, C., et al, 2020b).

On the other hand, SGC has shown bio-oil yields of up to 36% by weight, with a higher composition of phenolic compounds, fatty and nitrogenous acids; more abundant fatty acids were linoleic acid (38%), palmitic acid (19-34%), oleic acid (11%), and stearic acid (10%) (Primaz, C. T., et al, 2018; Ktori, R., Kamaterou, P., & Zabaniotou, A., 2018; Battista, F., et al, 2020). For its part, the SS under pyrolysis processes manages to extract up to 8.28% of saturated hydrocarbons, 6.69% of unsaturated aliphatics, and 7.77% of aromatic hydrocarbons from the liquid fraction obtained, and it found that it also presented high-value compounds such as caffeine, acetic acid, pyridine, and phenolics (dos Santos Polidoro, A., et al, 2018; del Pozo, C., et al, 2020).

Biofuels obtained from fermentable sugars

The high content of cellulose, lignocellulose, and lignin makes coffee residues, except mucilage, a raw material that requires pre-treatments to produce biofuels, these kind of pre-treatments are chemical, thermal, and enzymatic processes; A comparison of alkaline, acidic and thermal treatments (steam) to obtain fermentable sugars from CH reveals that as the acid or base concentration (H_2SO_4 and $NaOH$) increases from 0.5 to 2 M, there is an increase in the concentration of reducing sugars produced after the treatment with the enzyme cocktail, but the steam treatment favors the production of fermentable sugars by almost double when compared to the acid and alkaline methods (49% versus 22 and 26% respectively) (Shankar, K., et al, 2019; Montoya, A. C. V., 2019). The sugar profile obtained by HPLC analysis shows that for the steam treatment, the glucose content reached was 240 mg/g, xylose 161 mg/g, arabinose 31 mg/g, mannose 16 mg/g, and galactose 10 mg/g.

In comparison, SGC has reached sugar content of 58.2% for Mannose, 16% Glucose, 13% galactose, and 1.3% xylose, when they are subjected to an acid

pretreatment, followed by a delignification and finally the enzymatic treatment (Nguyen, Q. A., Cho, et al, 2019).

In the case of mucilage, which is a viscous residue composed mainly of fermentable sugars that can reach concentrations of up to 37.1 g / L in the case of glucose and 14.7 g / L for galactose, no post-treatment treatments are required simple centrifugation to eliminate most of the solids, unless Simple centrifugation to eliminate most of the solids unless there are compounds with the capacity to inhibit microbial growth such as phenolic compounds, alkaloids, and acids formed in the thermal degradation process (Orrego, D., et al, 2018; Kovalcik, A., Obruca, S., & Marova, I., 2018).

Due to this biochemical profile, different types of biofuels such as biomethane, biohydrogen, bioethanol, and biobutanol can be produced from hydrolyzed lignocellulolytic residues and coffee mucilage. The yields will depend on the number of fermentable sugars available, the type of microorganism, the operating conditions, and the inhibitory compounds present in the culture medium.

Bioethanol Production

Bioethanol (C_2H_5OH), can be used as a direct fuel or as a gasoline additive to increase the oxygenation of gasoline; it is a promising source of renewable energy and a promising substitute compared to most petroleum-derived fuels, producing fewer greenhouse effect after combustion. The coffee mucilage has allowed reaching final ethanol productions of 8.7 g / L through a co-culture of *Aspergillus niger* and *Saccharomyces cerevisiae* (Kousar, H., & Navitha, K. R., 2020).

Cultures used native strains of *Saccharomyces cerevisiae* have allowed obtaining a maximum ethanol concentration of 11.28 g / L from coffee mucilage (Do Viet, P., Le Pham, T. Q., & Le Nguyen, D. D., 2019).

Fermentations carried out by co-cultures of *Saccharomyces cerevisiae* subspecies allowed to obtain ethanol concentrations of 51.7 g / L from SGC (Dadi, D., et al, 2018). These results show the high potential for the production of bioethanol from the fermentable sugars obtained from SCG and those contained in the coffee

mucilage, reaching concentrations of bioethanol well above those reported in other works from other matrices (Atabani, A. E., et al, 2019).

Biobutanol Production

N-butanol is a compound mainly used as a solvent, extractant, a base product for the chemical and fuel industry. If a direct comparison with ethanol, it has a higher flash point, higher energy content, lower volatility, and lower hygroscopicity, and like ethanol, it can mix with gasoline to improve its octane number. The residues of the coffee husk (CH) have not been an exception when it comes to being used as renewable for the production of biobutanol and from the CH hydrolyzate and using *C. beijerinckii*, a butanol yield of 0.269 g / g of fermentable sugar (Hijosa-Valsero, M., et al, 2018).

The butanol produced from the silver skin of coffee (SSC) compared to bioethanol was 20 times higher; the maximum yield of glucose in biobutanol was 0.18 g/g (Procentese, A., et al, 2019). There are even reports of biobutanol yields of up to 0.31 g / g when there is hydrolysis before fermentation with *Clostridium beijerinckii* (Procentese, A., et al, 2018).

Biohydrogen Production

Among the most promising energy sources, hydrogen occupies the first place; its combustion energy is much higher than that of other fuels (122 kJ / gH₂) is 2.5 times higher than the obtained from hydrocarbons. Unlike the biofuels mentioned above, biohydrogen and biomethane require anaerobic fermentation conditions, and in some cases, production is higher in dark fermentations (Moreno Cárdenas, E. L., et al, 2020).

Under optimized production conditions, it has been possible to produce up to 3.04 L H₂ / Ld from the CH hydrolysate (Montoya, A. C. V., et al, 2020). Other authors have reported under this same pretreated substrate a yield of 86 NmLH₂ / g CH (dos Santos, L. C., et al., 2018).

On the other hand, other wastes such as coffee mucilage have combined with other organic wastes; It has made it possible to achieve a total volume of hydrogen

production of 129.95L / L when the co-culture of *Kocuria kristinae* and *Brevibacillus laterosporus* is used (Moreno Cárdenas, E. L., et al, 2019).

Biomethane Production

Coffee residues have a high content of carbon and nitrogen that makes them attractive raw materials for the production of biomethane; this is produced traditionally through the anaerobic digestion of organic matter and from the sludge generated in the processes of wastewater treatment (Kim, D., et al, 2018).

Coffee mucilage has allowed a very promising production of methane from bio-augmented manure inoculate, even reaching a yield of 294.5 L / Kg (Chala, B., Oechsner, H., *et al*, 2018). The coffee husk has also investigated in the production of methane in an anaerobic fermenter (UASB) after its previous treatment with ozone, and a yield of 86 mL of CH₄ / g of CH was achieved (dos Santos, L. C., et al., 2018).

Comparative studies of the methane-producing capacity from SGC and cow manure in anaerobic fermenters show a higher production from the coffee residue with a total accumulated volume of 1444 mL; for the second residue, the total methane was 1047 mL; also, the fractions corresponding to the gases generated from SGC were 53.7% methane and 37.8% CO₂, compared to the of cow manure that was 36.7% methane and 27.9% CO₂, which can favor large-scale gas separation processes from the products derived from coffee waste (Luz, F. C., et al, 2017).

Chapter 5

Other research contributions

5.1 Enzyme mimics in-focus: Redefining the catalytic attributes and implementing artificial enzymes.

Enzymes are proteins that possess the unique ability to accelerate the rate of chemical reactions up to 10^{19} times with an outstanding specificity. These powerful biological catalysts (biocatalysts) are essential to sustain life as they catalyze more than 5,000 biochemical reaction types in the cell. However, regarding industrial processes, their practical application has been limited by the low operational stability, harsh environmental conditions, and expensive preparation process. To address those limitations, artificial enzymes born as synthetic molecular complexes, based on amino acids or peptides, with the ability to mimic and surpass the catalytic activity of natural enzymes, with the plus of high stability and low production costs. This new field has created new possibilities for many industries as brewing, dairy, paper, molecular biology, and even energy, which is one of the most valuable resources. Currently, a global transition from fossil fuels to renewable and sustainable energy is urgently needed. Here, artificial enzymes can play a key role in the technological development required for the new generation of renewable energy, biofuels and biohydrogen. Thus, this work is a comprehensive study of the main considerations for the design of artificial enzymes and explores their promising potential as biocatalysts for renewable energy production (**Annex Figure 3**).

5.2 Multi-dimensional applications of nano-biomaterials in the medical sector of the modern world: Tissue engineering and COVID-19.

Over the past years, biomaterials-based nano-cues with multi-functional characteristics have been engineered with high interests. The ease in fine tunability with maintained compliance makes an array of nano-biomaterials supreme candidates for the biomedical sector of the modern world. Moreover, the multi-functional dimensions of nano-bio elements also help to maintain or even improve the patients' life quality most securely by lowering or diminishing the adverse effects of in practice therapeutic modalities. Therefore, engineering highly efficient, reliable, compatible, and recyclable biomaterials-based novel corrective cues with multipurpose applications is essential and a core demand to tackle many human health-related challenges, e.g. current COVID-19 pandemic.

Moreover, robust engineering design and properly exploited nano-biomaterials deliver wide-ranging openings for experimentation in the field of interdisciplinary and multidisciplinary scientific research. In this context, herein, we reviewed the applications on tissue engineering and therapeutics of

COVID-19 with the potential of several biomaterials. Following a brief introduction, drug delivery routes and mechanisms of biomaterials-based nano cues are discussed with suitable examples. The second half of the review focuses on the mainstream applications changing the dynamics of 21st century materials. In the end, current challenges and recommendations are given for a healthy and foreseeable future.

Final Conclusions and personal perspective

In Mexico, there is still a long way to go regarding the whole improvement of the current coffee production cycle. Nonetheless, the implementation of agroforestry systems and organic production schemes is a gigantic step to face this crisis. This work demonstrates the need for research in the country. It also provides a general overview of the techniques implemented worldwide, focused on efficient production, recycling economy and sustainable pest management.

Regarding CLR, as future work exists a big opportunity area of research based on the relationship between HV and native coffee varieties, both at the molecular level and as part of an ecosystem. On the other hand, more field studies are required since the degree of virulence of CLR also depends on the environmental conditions.

The sustainability and living conditions of coffee producers are also an important aspect, but even nowadays we do not have enough data to take the most suitable decisions, regarding ecological programs. Thus, more detailed topographic studies, models that estimate greenhouse gas emissions, as well as simulations that predict environmental conditions are required to identify on time the possible periods of the year that present greater instability in the rainfall and temperature patterns. This kind of analysis can allow the development of preventive strategies to reduce the losses of organic crops. Nonetheless, that should not be the only path. It is necessary to implement new policies, incentives for farmers, and new market models based on a sustainable approach to have a real impact.

On the other hand, coffee processing by-products, as coffee husk, have emerged as a promising solution for the removal of contaminants as caffeine. The main advantages of this proposal are its low production costs, it is reproducible and scalable, is safe, and above all, it would be adding value to a waste that negatively impacts the environment and the health of coffee producers. This approach could be used not just in water bodies, but in remote areas, in specific in hard-to-reach communities. This opens the opportunity to develop a new generation of bio-adsorbents, using coffee residues as raw material, such as the silver skin and

spent ground coffee, which have shown to be excellent bio-adsorbents for different types of heavy metals, colorants, and antibiotics. Thus, adding value to residues will generate new production processes, with a neutral or positive impact to our environment.

In the end, the crisis of coffee production is a complex problem that goes beyond a pest or a waste accumulation. It involves social, economic, and political concern due to the current situation of coffee producers. Technological development, updated regulations, and research will be crucial to conserve coffee biodiversity, but we need to act now, before reaching a point of no return where high mountain coffee production will be no more than a cultural memory. This ancient tradition, besides of being sustainable, is also the basis of our Mexican coffee.

Annexes



Figure 1. Varieties donated from coffee-growing communities, in Jaltenango de la Paz, Chiapas. We took samples of the most representative varieties cultivate in Jaltenango, which were *Arabe*, *Bourbon*, *Pache Criollo*, *Pacamara* and *Peñasco*. For those varieties we choose two varieties that presented the most contrasting behavior in their interaction with *Hemileia vastatrix*. In this case, *Arabe* was selected due to their highly susceptibility and because of their economical relevance in the market. Figure **1.A** shows a 15-years-old plant infected in the fields and **1.B** shows a 2-years-old plant infected under controlled conditions. On the other hand, the variety catalogue as *Peñasco* was selected because this variety is considered also susceptible; However, we observed a certain level of resistance developed not only in the field, but also under controlled conditions. Figure **1.C** shows a 15-years-old plant infected in the fields and **1.D** shows a 2-years-old plant infected under controlled conditions.

Experimental Design

Study of the Infection Process

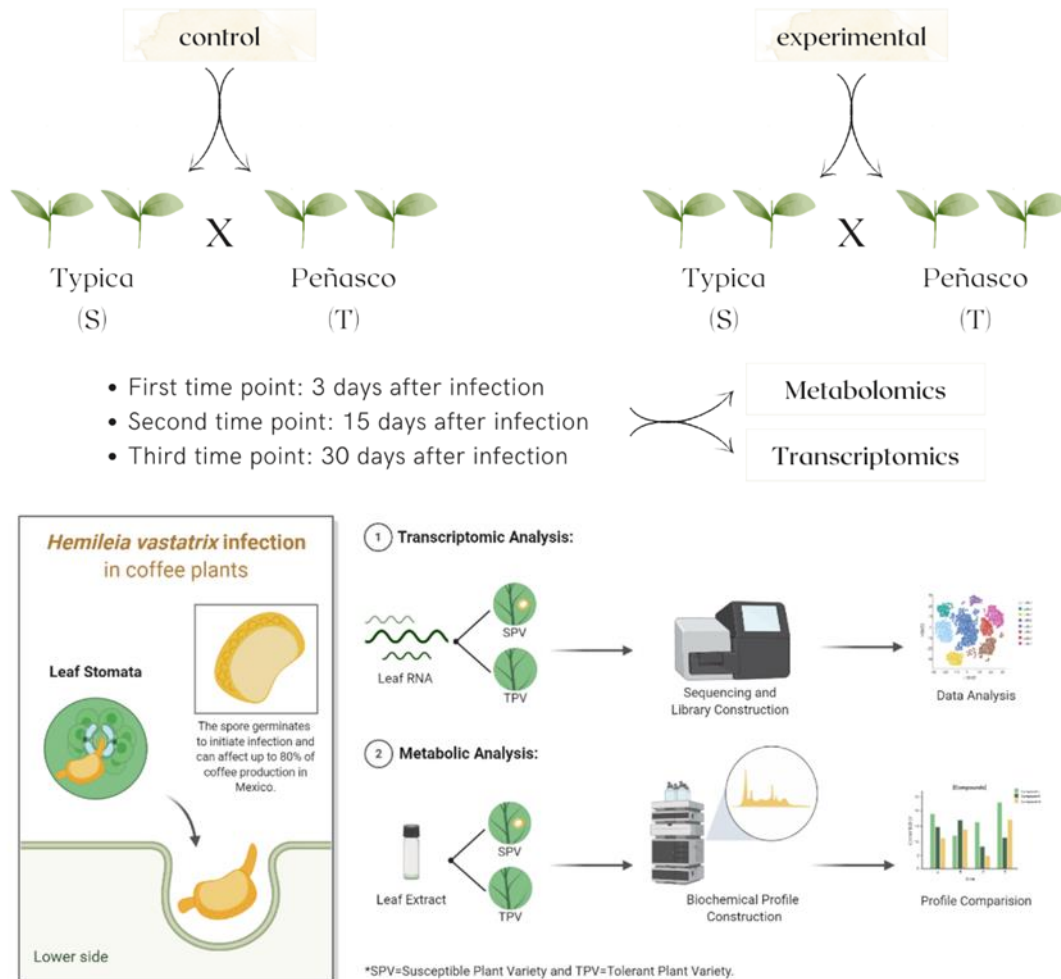


Figure 2. Experimental design for each point of time. Four leaves for each plant were taken, two for the RNA extraction required for transcriptomic analysis, and two for HPLC analysis for the metabolomic profile.

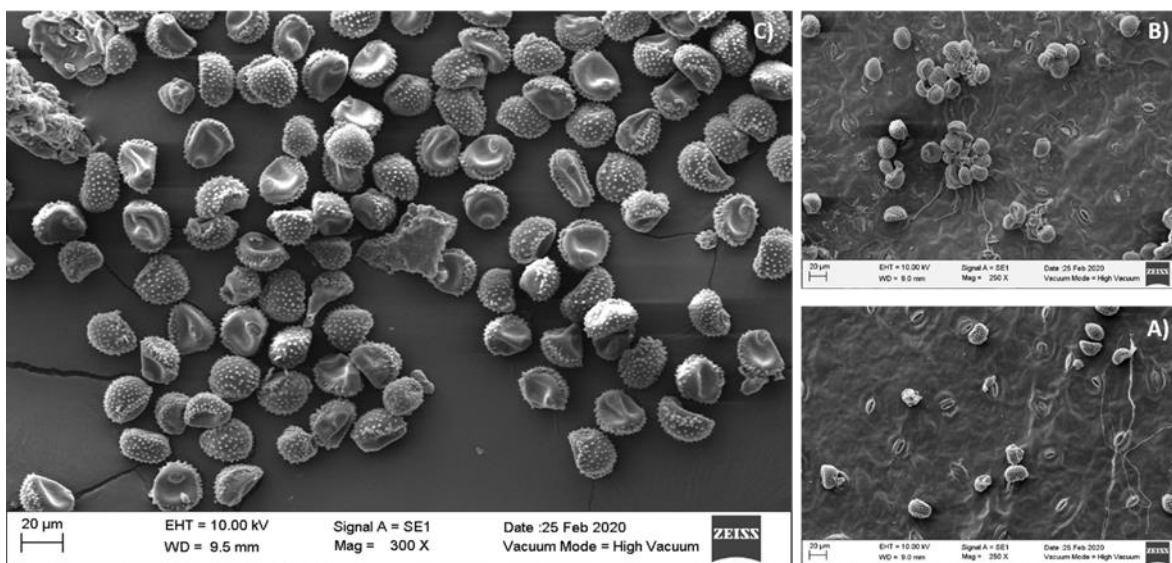


Figure 3. Evaluation of different levels of severity of coffee rust injuries in coffee leaves.

Artificial Enzymes

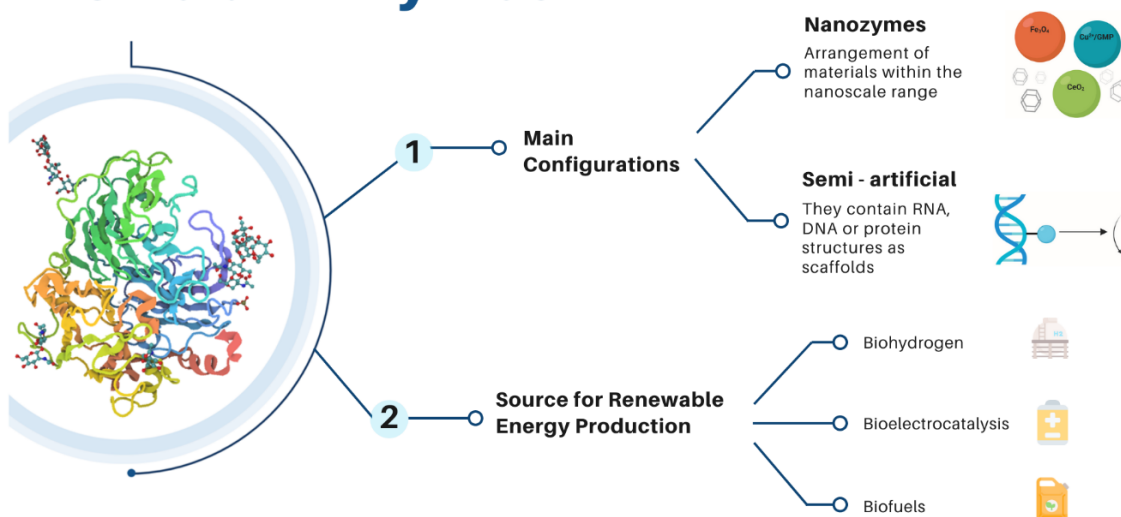


Figure 4. Visual Abstract of Enzyme mimics in-focus: Redefining the catalytic attributes and implementing artificial enzymes. Under revision by the *International Journal of Biological Macromolecules*.

Curriculum Vitae

Nora Esther Torres Castillo was born in Nuevo Laredo, Tamaulipas, México, on August 15, 1995. She earned the Biotechnology Engineering degree from the *Instituto Tecnológico y de Estudios Superiores de Monterrey*, Monterrey Campus in December 2018. Also, in August 2018, she obtained a double certification as Green Belt for the Lean Six Sigma program by *Tecnológico de Monterrey* and *Arizona State University*. She was accepted in the graduate programs in Biotechnology in January 2019.

Publications

- Castillo, N. E. T., Melchor-Martínez, E. M., Sierra, J. S. O., Ramirez-Mendoza, R. A., Parra-Saldívar, R., & Iqbal, H. M. (2020). Impact of climate change and early development of coffee rust—An overview of control strategies to preserve organic cultivars in Mexico. *Science of The Total Environment*, 140225 (**Published**).
- Melchor-Martínez, E. M., Castillo, N. E. T., Macias-Garbett, R., Lucero-Saucedo S., Parra-Saldívar, R., & Sosa-Hernández, J. E. (2020). Multi-dimensional applications of nano-biomaterials in the medical sector of the modern world: Tissue engineering and COVID-19. *Frontiers* (**Accepted**).
- Castillo, N. E. T., Sierra, J. S. O., Melchor-Martínez, E. M., Ramírez-Torres, N. M., Parra-Saldívar, R., & Iqbal, H. M. (2020). Enzyme mimics in-focus: Redefining the catalytic attributes and implementing artificial enzymes. *International Journal of Biological Macromolecules* (**Under revision**).
- Castillo, N. E. T., Sierra, J. S. O., Melchor-Martínez, E. M., Parra-Saldívar, R., & Iqbal, H. M. (2020). Adding value to coffee husk as a bio-adsorbent to tackle caffeine emerging contaminant – A minireview. *Case Studies in Chemical and Environmental Engineering* (**Under revision**).
- Castillo, N. E. T., Sierra, J. S. O., Melchor-Martínez, E. M., Parra-Saldívar, R., & Iqbal, H. M. (2020). Coffee processing by-products as potential low-cost substrates for Bioenergy production – A minireview. *Bioresource Techonology* (**Under revision**).
- Castillo, N. E. T., Donjuan-Montoya, K.A., Melchor-Martínez, E. M., Parra-Saldívar, R., & Iqbal, H. M. (2020). Defining the meaning of being a small coffee producer in Mexico - A case study of high mountain coffee produced in Jaltenango, Chiapas. *Crop protection* (**Under revision**).

Research Calls

- Novartis, Hello science, 2020 → No answer
- Bayer Crop Science, HALO: Fighting pests while preserving biodiversity, December 2020
→ Pending results
- PRONACES – CONACYT, Socioecological Systems, January 2021 → Pending results

Awards

- Cemex Tec Award, *transforming communities* (August 2020) → Winners
- HULT Prize (June, 2021) → Pending results
- Rómulo Garza Award (February, 2021) → Pending results

Other scientific dissemination activities

- Guest to the panel discussion *Primero un café, luego existo* organized by the scientific social media group LabGeek México (July 2020).
- Participant in the podcast *echale coco* for the chapter *Café en México* (December 2019).
- Organizer of the coffee tasting course *Por amor al café mexicano* (November 2019).
- Coordination assistance and host of the workshop *Facilitating evidence-based decision-making in global sustainability* for the Global consortium for sustainability outcomes (GCSO) (June 2019).
- Co-coordinator of *Sustainable and Applied Biotechnology* seminar (February 2019).

This document was typed in using Microsoft Word by Nora Esther Torres Castillo.