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Escuela de Ciencia Biológicas e Ingeniería

A yeast-based cytotoxic assays of commonly used fungicides in Ecuador

Trabajo de integración curricular presentado como requisito para la obtención del título de Biólogo

Autor:

RONQUILLO ZAMORA ROBERT ANTONY

Tutor:

Fernando Alexis Gonzales Zubiate, PhD.

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Dedicatoria

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Resumen

Los fungicidas controlan o previenen infecciones causadas por hongos fitopatógenos en muchos cultivos importantes a nivel mundial. Epoxiconazol (EPO), Fenpropimorph (FEN), Mancozeb (MZ) y Pyrimenthanil (PYR) son los fungicidas más utilizados contra la Sigatoka Negra (SN) en la producción de banano. La Sigatoka Negra infecta las hojas de banano, lo que provoca una reducción de la capacidad fotosintética de la planta y, en última instancia, conduce a la muerte de las hojas, lo que limita drásticamente la producción de banano. En Ecuador, así como en todos los demás países productores de banano, la Sigatoka negra se controla principalmente mediante varias aplicaciones aéreas de fungicidas de EPO, FEN, MZ y PYR en formulaciones individuales o en cócteles. Si bien varios autores han señalado los efectos toxicológicos de EPO, FEN, MZ y PYR en el organismo modelo *Saccharomyces cerevisiae*, los productores de banano siguen aplicando grandes cantidades de estos fungicidas en miles de kilómetros de este cultivo. En Ecuador, existe una falta de información sobre los riesgos toxicológicos de estos químicos para los trabajadores bananeros y las comunidades que viven en las plantaciones bananeras. Por lo tanto, esta revisión de la literatura describe la biología y el manejo actual de BLSD con formulaciones comerciales en Ecuador. Además, se representan análisis citotóxicos sobre los efectos nocivos sobre *S. cerevisiae* para proporcionar información sobre las posibles afectaciones en la salud de las personas que viven cerca de las plantaciones de Musaceas.

Palabras claves: *Saccharomyces cerevisiae*, Fungicidas, Epoxiconazol, Fenpropimorf, Mancozeb, Pyrimenthanil, Enfermedad de la hoja de Sigatoka Negra, Ecuador, Producción de banano.

Abstract

Fungicides control or prevent infections caused by phytopathogenic fungi in many important crops worldwide. Epoxiconazole (EPO), Fenpropimorph (FEN), Mancozeb (MZ) and Pyrimenthanil (PYR) are the most used fungicides against Black Sigatoka Leaf Disease (BLSD) in banana production. BSLD infects banana leaves, causing a reduction of the plant's photosynthetic capacity and ultimately leading to the death of the leaves, drastically limiting banana production. In Ecuador, as well as all other banana producer countries, Black Sigatoka is mainly controlled by several aerial fungicides applications of EPO, FEN, MZ and PYR in solo or cocktails formulations. Even though several authors have pointed out the toxicological effects of EPO, FEN, MZ and PYR on the model organism *Saccharomyces cerevisiae*, banana producers heavily apply a tremendous amount of these fungicides over thousands of kilometers of this crop. In Ecuador, there is a lack of information about the toxicological risks of these chemicals for banana workers and communities living in banana plantations. Therefore, this literature review depicts the biology and the current management of BLSD with commercial formulations in Ecuador. In addition, cytotoxic analyses about the deleterious effects on *S. cerevisiae* are depicted to provide insights into the potential affectations on people's health living near Musa plantations.

Keywords: *Saccharomyces cerevisiae*, Fungicides, Epoxiconazole, Fenpropimorph, Mancozeb, Pyrimenthanil, Black Leaf Streak Disease, Black Sigatoka, Ecuador, Banana production.

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Abbreviations

AEBE	Asociación de Exportadores de Banano del Ecuador
AGROCALIDAD	Agencia de Regulación y Control Fito y Zoosanitario
BS	Black Sigatoka
BLS	Black Sigatoka Streak
BLSD	Black Sigatoka Streak Disease
BWG	Banana Working Group
DBCP	Dibromochloropropane (1, 2-dibromo-3-chloropropane)
DMI	Demethylation Inhibitors
EBDC	Ethylene-bis-di-thiocarbamate
EPO	Epoxiconazole
FENACLE	Federación Nacional de Trabajadores Agroindustriales, Campesinos e Indígenas Libres del Ecuador
FHIA	Fundación Hondureña de Investigación Agrícola
FRAC	Fungicide Resistance Action Committee
INEC	Instituto Nacional de Estadística y Censo
INIAP	Instituto Nacional de Investigaciones Agropecuarias
IPM	Integral Pest Management
MAGAP	Ministerio de Agricultura, Ganadería, Acuacultura y Pesca
MAATE	Ministerio del Ambiente, Agua y Transición Ecológica
MSP	Ministerio de Salud Pública del Ecuador
MZ	Mancozeb
PPE	Personal Protective Equipment
PYR	Pyrimethanil
WHO	World Health Organization

1. Introduction and Justification

Fungicides are pesticides used to control and mitigate phytopathogenic fungi in crops worldwide (Zubrod *et al.*, 2019). Fungicide application for controlling fungal diseases is considered indispensable to securing the global food supply of commodities. In wine-growing regions, fungicides account for over 90% of all pesticide applications. Likewise, fungicides constitute the primary chemical strategy in bananas to combat destructive infections such as Black Sigatoka Streak Disease (BLSD).

The cultivation of bananas constitutes the most important agricultural activity in Ecuador. According to INIAP (2021), one-third of all the exported bananas in the world originate from Ecuador. Furthermore, Ecuadorian bananas are exported to many regions of the world, including the European Union (42%), the USA (21%), Russia (20%), and 11% to the Mid-East, Northern Africa, Asia, and 6% to other Latin American countries. The Association of Banana Exporters of Ecuador (AEBE) highlights that the revenues generated by banana activity represent 3.84% of the country's GDP, 50% of the total agricultural GDP, and 20% of all the Ecuadorian exportations. Moreover, the cultivation of bananas and its adjacent industries employed more than 1 million families representing approximately 17% of the nation's total population. Thus, banana production is crucial for sustaining local food security and the local and international economies.

Black Sigatoka Streak Disease (BLSD), *Mycosphaerella fijiensis* Morelet (teleomorph), or *Pseudocercospora fijiensis* (anamorph), was first described in Fiji Islands in 1963 (Crous et al., 2011; Ploetz et al., 2003). From the South Pacific region, BLSD spread and infected all the banana-growing regions of Africa by late 1980 and commercial plantations of banana in America by 1990. The causative agent of BLSD is a harmful fungus that provokes up to 50% yield losses during the plant crop cycle (Stover, 1983; Pasberg-Gauhl, 1989; Mobambo et al., 1993). In Ecuador, Black Sigatoka was officially reported in 1987 in Esmeraldas province, located in the northern part of Ecuador. After two years, Guayas and Los Ríos presented the pathogen in their plantations. By 1992, the Black Sigatoka reached the southern part of Ecuador in the province of El Oro, covering all the plantations of *Musa* spp in the country. The current strategies to control BLSD worldwide include using chemical fungicides and cultural management (defoliation of infected leaves, drainage, soil nutrition, irrigation methods, and

pest control) to mitigate or maintain low infection rates in commercial and non-commercial banana plantations.

In Ecuador, banana farmers mainly utilize Protectants (Mancozeb) and Systemic (amines, anilinopyrimidines, and triazoles) fungicides as part of the management program for BLSD, applying them in solo or cocktail mixtures (INIAP, 2021). Aerial spraying heavily applies these formulations in commercial banana plantations in Ecuador's coastal region. Several studies in commercial plantations have pointed out that these formulations mostly contain the following fungicides Epoxiconazole, Fenpropimorph, Mancozeb, and Pyrimethanil (Portilla 2017, Santillan 2017). Nonetheless, all the fungicides mentioned above severely damage biological systems. Thus, several authors treated mice with conazoles (epoxiconazole) and found that they responded with increased hepatomegaly, increased metabolism of all-trans-retinoic acid (atRA), increased oxidative stress, and decreased hepatic levels of atRA, decreased serum cholesterol levels, and increased hepatic cell proliferation. (Allen et al., 2006; Bruno et al., 2009; Chen et al., 2009; Juberg et al., 2006; Nesnow et al., 2009, 2011a; Ortiz et al., 2010; Peffer et al., 2007; Ward et al., 2006). Likewise,
Environmental Protection Agency (EPA) and the World Health Organization (WHO) have raised concerns about Mancozeb's long-term toxicity associated with endocrine, mutagenic, carcinogenic and teratogenic risks (WHO, 1988; EPA, 2005). On the other hand, Gupta (2018) stated that Fenpropimorph is a mild irritant to rabbit skin and is associated with developmental toxicity and malformations in the same organism. Finally, Pyrimethanil assayed in rats can produce thyroid follicular cell tumors and enhance hepatic thyroid hormone metabolism, which may be responsible for thyroid tumorigenesis.

Despite intensive use of fungicides and the associated potential ecotoxicological and health risks, fungicides' environmental fate and effects have received far less attention than insecticides and herbicides (Zubrod, 2019). Banana production activities are linked with severe and uncontrolled fungicide applications applied annually to protect the crop from BLSD. This activity has led to several side effects of pathogen resistance, fungicide residue, environmental pollution, and health conditions in workers and communities near these plantations (Brisbois, 2016).

2. Problem Statement

Our modern societies demand more goods to supply the increasing demand of an exponentially growing human population. As a result, agricultural practices worldwide have turned over the use of pesticides to guarantee the continuous supply of important staple food for domestic and international consumption. Within this group of pesticides, fungicides arise as protectors of many products that range from cereals to fruits commercialized for billions of dollars annually. Nonetheless, using these fungicides to prevent or combat many destructive fungal infections has also caused a series of critical hazards to the environment, animals, and humans.

Using fungicides to combat pathogenic fungi has been reported to cause several dangerous side effects. Fungicides' side effects are related to soil contamination, water bodies contamination, food persistence, intoxication, and death of humans. As an agricultural hotspot of many renowned products, most farmers use fungicides to combat harmful fungi in Ecuador. Specifically, banana production requires abundant fungicides to combat Black Sigatoka caused by *Mycosphaerella fijiensis*. The main fungicides used to control this disease are Epoxiconazole, Fenpropimorph, Mancozeb, and Pyrimethanil which are applied in solo or cocktails mixtures through terrestrial and aerial methods for more than tenth cycles over a year. However, the use of the fungicides mentioned above has raised concerns about the side effects they provoke in many mammalian systems regarding the concentrations, application methods, and disposal measurements after the usage.

Therefore, this literature review is intended to present a detailed panorama of the deleterious effects of four banana fungicides used in banana production on the yeast *Saccharomyces cerevisiae*. Additionally, the current management of BLSD with commercial fungicides in Ecuador is depicted to provide a general scene of the side effects of these chemicals that mainly affect banana workers and communities living near plantations. Thus, these cytotoxic analyses and the facts of fungicide management are intended to provide insights into the excessive use of commercial fungicides and their potential affectations on people's health close to Musa plantations.

3. Objectives

3.1. General Objective

Elaborate an orderly and informative analysis of the physiologic, genetic, proteomic, and transcriptomic effects of *Saccharomyces cerevisiae* under four common banana fungicides used against Black Sigatoka Streak Disease in Ecuador.

3.2. Specific Objectives

To describe the history, biology, and epidemiology of Black Sigatoka Streak Disease.

To exhibit the current situation in Ecuador related to banana fungicide management in the agricultural sector.

To report the physiological, genetic, transcriptomic, and proteomic responses of Epoxiconazole, Fenpropimorph, Mancozeb, and Pyrimethanil in *S. cerevisiae* metabolism.

To describe the *S. cerevisiae* mechanisms of toxicity elicited by fungicides (Epoxiconazole, Fenpropimorph, Mancozeb, and Pyrimethanil) and their correlation with human cells.

4. Yeast

4.1. Description and Classification of Yeasts

Yeasts are unicellular microorganisms that belong to the Fungi kingdom. Most of them utilize organic compounds (simple hexose sugars) as an energy source and do not require sunlight to grow (Ebahi et al., 2013). Generally, yeasts prefer a 25-30 °C temperature range to grow, but some species can also grow in a temperature range between 0-47 °C. Moreover, these microorganisms can develop under aerobic or anaerobic conditions depending on the species. They reproduce by budding and occasionally by fission and do not form spores in or on a fruiting body. The yeast cell cycle typically has four phases (G1, S, G2, and M), and its regulation is similar to higher eukaryotes. *Saccharomyces cerevisiae*, a model organism, can undergo budding, conjugation, and sporulation, as depicted in Figure 1.

Numerous criteria are considered for classifying these organisms, including physiology, cell morphology, immunology, and molecular biology. Nonetheless, the more accurate method used to classify them lies in molecular biology, which uses sequence analysis to classify the new species. (Walker, 2009). Researchers have categorized 100 genera of yeasts under three main fungal kingdoms Ascomycotina, Basidyomicotina, and Deuteromycota. Around 1500 species of yeasts are described; however, new species are regularly characterized (Walker, 2009).

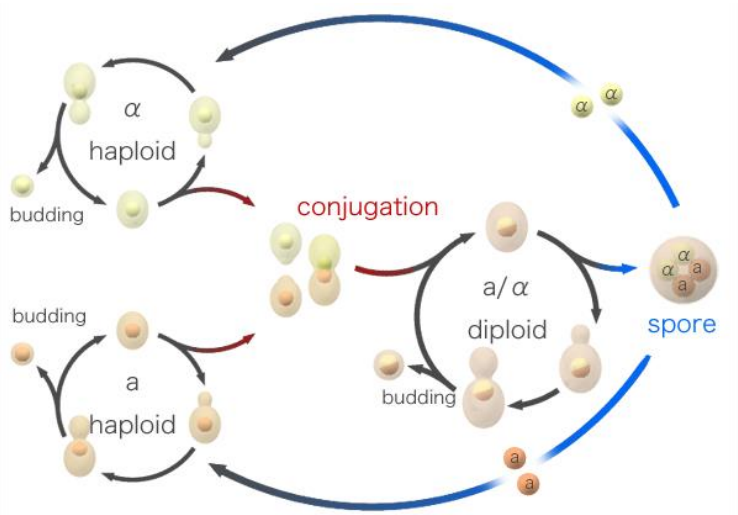

Figure 1. *Saccharomyces cerevisiae* cell cycle. Image available from Wikimedia Commons.

4.2. Yeast Ecology

Yeasts occur in many habitats such as soil, water, plants, and insects. Most yeasts have a suitable environment in plant tissues (leaves, flowers, and fruits), and a small proportion develops commensal or parasitic relationships with animals. Yeasts are adaptable organisms that can develop in harsh conditions like high salt/sugar concentration, low temperatures, and low oxygen availability.

Yeasts performed essential roles in the food chain. For example, ascomycetous yeasts transformed low-molecular-weight nitrogenous compounds into proteins beneficial to insect nutrition. Besides, several insect species, especially *Drosophila* spp., feed on yeasts that colonize plant material. Filter feeders also use yeasts as a source of an ailment.

4.3. Yeast Cell Structure

Yeast cell size varies enormously depending on the species and growth conditions. For example, the shortest yeasts have 2-3 um length while others can achieve 20-50 µm lengths. On the other hand, cell widths do not represent significant variation; they are around 1-10 µm. Furthermore, yeasts have characteristic pigments on their surfaces, for example, white, black, cream, yellow, orange, and red. Some of these pigments have applications in biotechnology (astaxanthin pigment from *R. rhodozyma*).

4.4. Applications

Fermentation to produce bread, beer and wine has been the principal usage of yeasts for thousands of years. *Saccharomyces cerevisiae* is the most exploited microorganism for potable and industrial ethanol production, representing the number one biotech commodity worldwide. Nonetheless, other non-*Saccharomyces* species are now used to produce other vital commodities. Yeast also plays an essential role in biotechnology's environment and health care sector. Furthermore, yeasts are studied for the development of biosensors due to their practical aspects of handling (low cost; ease of cultivation, growth, transport, conservation, storage, and genetic manipulation), [2] conserved biological processes in mammals, and [3] the potential to serve as bioremediation, toxicity and genotoxicity agents (Mollocana, 2020).

5. Fungicides

5.1. History of fungicides

The history of fungicides can be divided into three eras: The First or Sulfur Era, The Second or Copper Era, and The Third or Organic Fungicide Era. The Sulfur Era started in ancient times and finished in 1882. Then, the Copper Era from 1882 to 1934, and the third era from 1934 to nowadays (McCallan, 2012).

Fungicides are as ancient as agriculture. The Homeric poems (8 BC) depicted the purifying nature of sulfur used as a cleansing agent or disinfectant for sacred objects and places. Thus, this constitutes the first reference to using a substance as a pesticide and maybe even the first fungicide. (McCallan, 2012). Since ancient times, the most important diseases affecting human crops have been smut and rust of grains. So it is not surprising that Pliny the Elder around I BC reported in his 'Natural History' the steeping of seed grain in wine or a mixture of cypress for controlling mildew that probably was smut. By 1756, Aucante used treatment against smuts that included lime, arsenic, and corrosive sublimate and reported the effectiveness of these ingredients.

In 1776, Schulthess used copper sulfate as a treating medium against the bunt. Then, Prévost evaluated the effect of copper sulfate at different concentrations, temperatures, and duration of treatments on the viability of the spores of the bunt fungus. Nevertheless, Julius Kuhn was the first to elaborate a recommendation specifically describing a solution of 0.5% copper sulfate to immerse grains for 12 to 14 hours to control bunt fungus (Woolman and Humprey, 1924; McCallan, 2012).

Up to the XIX century, sulfur, mercury, arsenic, copper sulfate, zinc chloride, and glycerides (amurca and dregs of olive oil) were fungicidal chemicals. Nonetheless, Horsfall (1956) stated that just two of the compounds mentioned above act appropriately as fungicides: copper sulfate and zinc chloride (McCallan, 2012). He argued that the four left was probably first known as insecticides. In 1847, Edward Tucker successfully applied sulfur as a remedy for grape seeds powdery introduced in England, possibly from America. However, the disease spread into France, and Duchatel applied sulfur dust on grape leaves moistened with dew. This application represents the first fungicide dusting application. Nonetheless, the Eau de Grison

or Grison's Liquid, a mixture made by Grison, the head gardener of the vegetable houses of Versailles, became the most notable of the early lime sulfur preparations. Therefore, this mixture was used in fruit trees, grapes, and hops to control powdery mildews and peaches for leaf curl, while copper sulfate was used as a steep to control wheat bunt.

By 1870, Downy mildew, causative agent *Plasmopara viticola*, threaded the wine industry in France. Finally, after six years, the fungal infection was controlled thanks to the Bordeaux mixture, a preparation developed by Millardet, a prominent French botanist, aided by chemist Gayon. Bordeaux mixture was developed based on observations made by Milladert of the use of a rudimentary mixture of lime and copper sulfate to discourage petty thieving of wine in the roadway in Gironde. His experiments concluded with a 'Bordeaux' formula (3 parts of copper sulfate and 1 part of calcium oxide to 100 parts of water) adopted in France.

Several rival preparations were also released to compete with the Bordeaux mixture. The first one was released in 1886 and substituted ammonia for lime, and it was called Eau Celeste. A year later, the soda mixture or Burgundy mixture used sodium carbonated instead of lime, but it had a modest success due to being not too caustic as Eau Celeste on wine leaves. Finally, the modified Eau Celeste, a combination of the former, had limited success. For that reason, Chester modified in 1891 that dissolved copper carbonate in an ammonium carbonate solution.

Bordeaux mixture was greatly appreciated by its undoubtedly fungal efficiency; however, its fungal activity causes significant damage to the foliage of many plants. Therefore, an increasing interest in finding a less toxic form of copper resulted in the so-called fixed-copper fungicides developed mainly by the industry from 1920 to 1930. Fixed-copper fungicides represented a partial solution because their fungitoxic activity was sacrificed by a more soft phytotoxicity performance. So, fixed-copper fungicides can be classified into four categories: (1) the primary sulfates, (2) the basic chlorides, (3) the oxides, and (4) various groups of copper silicates, phosphatases, and zeolites (McCallan, 2012).

Nowadays, the Bordeaux mixture has lost the title of universal fungicide, firstly due to the reintroduction of lime sulfur in 1906, then by copper-fixed fungicides, and the final stroke with the introduction of dithiocarbamates. Nonetheless, this mixture is currently used as a specialty fungicide, especially in the US and several countries.

The third era was characterized by the development of highly-specific agents, i.e., organic fungicides, and increasing public demand for improving evaluation methods that assess precision in vitro and the field and consequently the effects of the fungicide on the animals, and humans, and the environment (McCallan, 2012).

In 1934, the first dithiocarbamates, derived from dithiocarbamic acid, were registered in a patent for 'disinfectants' to control and prevent the growth of bacteria and fungi by Tisdale and Williams, assignors of Du-Pont de Nemours and Co. Dithiocarbamic acids such as tetramethyl thiuram had previously been known as rubber accelerators. In 1937, at Delaware Agricultural Experiment Station, Guy reported tetramethyl thiuram disulfide (thiuram) insecticidal properties against adult Japanese beetles. After three years, Muskett and Colhoun experimented with thiuram and reported its activity as a seed dressing agent protecting flax seeds against seedling blight. Since then, tetramethyl thiuram disulfide has been known as one of the most successful and universal seed treatment chemicals. Nonetheless, its widespread use as a spray protectant, especially in the US, suffered a ten-year delay due to the reports of dermatitic effects on a few sensitive individuals. Thus, from 1950, the properties of thiuram were massively used in the United States to control turf leaves and other diseases. Another important dithiocarbamate was Ferbam, formerly named Fermate in 1940, consisting of three methyldithiocarbamate groups attached to ferric iron. This compound was almost specific for controlling anthracnose diseases and apple rust. In addition, it obtained great acceptance as a spray for ornamentals due to its low phytotoxicity compared to copper and sulfur sprays. Finally, Ziram, zinc diethyldithiocarbamate, achieved good fame when applied to control early blight in tomatoes and potatoes.

The dithiocarbamates mentioned above were only prepared with a monoamine and a disulfide. However, W. F. Hester prepared a new compound, disodium ethylenebisdithiocarbamate (diethane), from a diamine. In the beginning, Diethane, later Nabam, showed high instability and a controversial non-fungicidal effect. Nevertheless, the addition of zinc sulfate made it stable, and it became a new fungicide known as 'Zineb' that was widely used for potato diseases, almost replacing the current-widely-spread Bordeaux mixture in the US. Finally, Maneb, manganese ethylenebisdithiocarbamate, was first tested in potato crops in 1946. Though it represents the latest test, it became the most effective dithiocarbamate protecting a broad spectrum of vegetable crops against many diseases.

Systemic fungicides began in 1966 with the development of oxanthins, which are efficient mainly for controlling carbons and rusts. In 1984, phenylamides, which are specific for Phycomycetes, were introduced. In 1988, benzimidazoles appeared, fungicides efficient against fungi of the Deuteromycetes, ascomycetes, and basidiomycetes groups. In 1988, ergosterol inhibitors were also developed, which control the same groups of fungi as the benzimidazoles. In the late 1980s, strobilurins were synthesized, derived from natural

compounds produced by fungi of the order Agaricales, which are fungicides that control most fungal groups.

5.2. Use of fungicides

Fungicides are pesticides that prevent or eradicate fungal infections from plants or seeds (Gupta, 2018). In general, fungicides are used: [1] to control diseases during the establishment and development of a crop, [2] to increase crop productivity and reduce blemishes, and (c) to improve the storage life and quality of harvested plants and produce. Some of the most significant disease losses occur post-harvest (McGrath, 2004). Fungi often spoil stored fruits, vegetables, tubers, and seeds. A few which infect grains produce toxins (mycotoxins) capable of causing severe illness or even death in humans and animals when consumed. Moreover, fungicides have been used to reduce mycotoxin contamination in wheat affected by Fusarium head blight; however, most fungicides have not been sufficiently compelling for managing mycotoxins associated with other diseases.

A fungicide should possess the following properties to exert its action correctly: (1) high toxicity to the particular fungus but low toxicity to the plant/animal; (2) activity per se or the ability to be converted (by plant or fungal enzymes) into toxic intermediates; (3) the ability to penetrate fungal spores or the developing mycelium to reach the site of action; (4) low ecotoxicity; and (5) the ability to form a protective, consistent deposit on the plant surface that helps it resist atmospheric conditions as sunlight, rain, and wind (Phillips, 2001; Gupta, 2008). Fungicides account for more than 90% of pesticide applications in wine-growing regions (Zubrod et al., 2019).

5.3. Classification of fungicides

Fungicides have different classification systems, as is shown in Figure 2. In Latin America, World Health Organization (WHO) classification system is broadly used (WHO). This system is based on the toxicity grade displayed by a substance (Mollocana, 2020). Furthermore, Gupta (2018) has grouped fungicides according to their chemical structure into nine groups, and this designation facilitates fungicide studies because the mechanisms of action may be related (Figure 8).

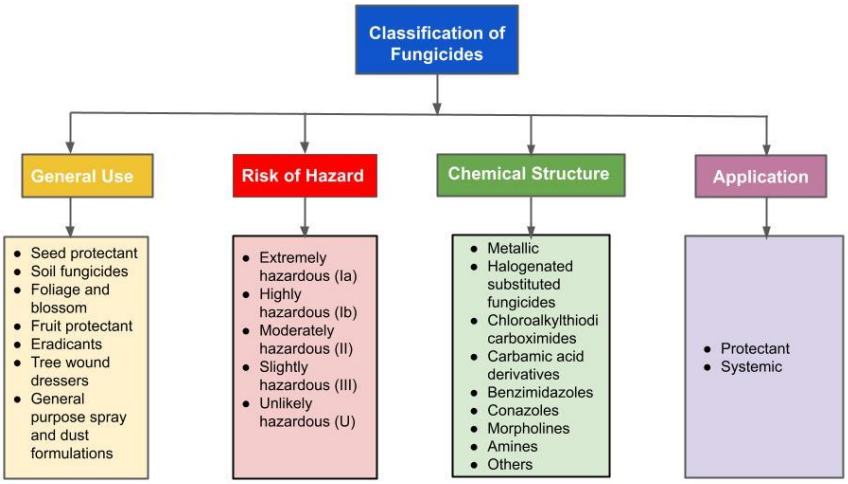

Figure 2. Classification of Fungicides according to distinct parameters. Adapted from Mollocana 2020.

5.3.1. Epoxiconazole

Epoxiconazole belongs to triazole (conazole) fungicides, belonging to Demethylation Inhibitors DMI-fungicides (Class I), which have been used worldwide for control of a variety of plant pathogens as well as human and animal antimycotic therapy (Yan et al., 2015; FRAC, 2021) (Figure 3). Regarding its properties against many fungi such as Ascomycetes, Basidiomycetes, and Deuteromycetes in several economically important crops: cereals, grapes, fruits, and vegetables (Li et al., 2015). Epoxiconazole was manufactured for the first time by the BSAF Company. According to Yan and co-workers (2015), the fundamental biological mechanism of all azo le compounds is the inhibition of the synthesis of the steroid ergosterol, an essential membrane component in fungi. Specifically, conazole fungicides were designed to inhibit both cytochrome P450 (CYP) (Erg11) enzyme and CYP51 (lanosterol-14α-demethylase) required for catalyzing demethylation in ergosterol biosynthesis (Turi and Loper, 1992; Georgopapadakou and Walsh, 1996; Zarn et al., 2003), leading to altered integrity of plasma membranes and a high content of fatty acids (Van den Bossche et al., 1983) (Figure 4).

A study performed by Guan and collaborators (2020) about functional genome-wide knockout mutant profiling with five conazoles (Propiconazole, Penconazole, Tebuconazole, Epoxiconazole, and Flusilazole) in *Saccharomyces cerevisiae* reconfirmed that conazoles have similar responsive gene patterns and molecular toxicity mechanisms that specifically influence plasma membrane integrity. Furthermore, they found that some DNA damage-associated pathways support the hypothesis of the potential carcinogenic properties of conazoles.

In mammalian systems, most conazoles can both induce and inhibit hepatic CYPs. Several authors treated mice with conazoles and found that they responded with increased hepatomegaly, increased metabolism of all-trans-retinoic acid (atRA), increased oxidative stress, decreased hepatic levels of atRA, decreased serum cholesterol levels, and increased hepatic cell proliferation. (Allen et al., 2006; Bruno et al., 2009; Chen et al., 2009; Juberg et al., 2006; Nesnow et al., 2009, 2011a; Ortiz et al., 2010; Peffer et al., 2007; Ward et al., 2006). The more negative effects have been reported to cyproconazole, epoxiconazole, and propiconazole inducing hepatocellular carcinomas and hepatocellular adenomas in mice. (Hester et al., 2012).

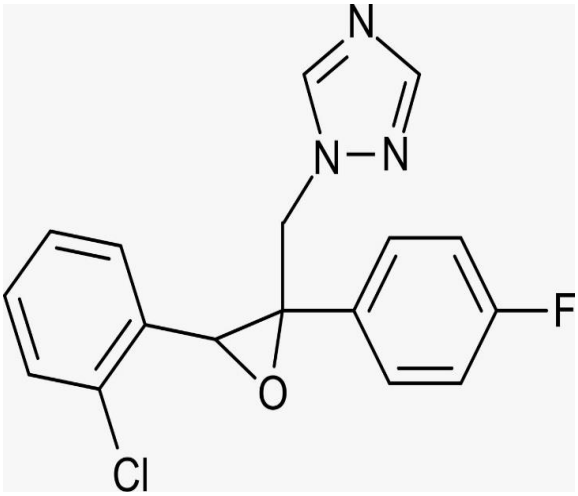

Figure 3. Graphical representation of the fungicide Epoxiconazole. Image available from Wikimedia Commons.

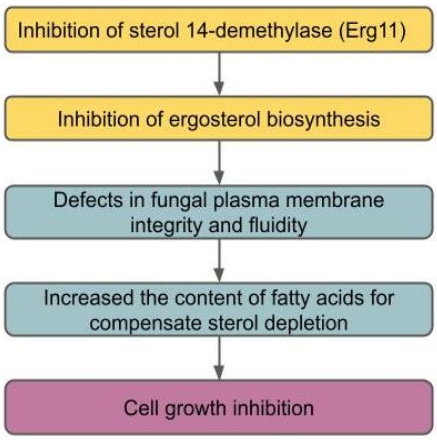

Figure 4. General Mechanism by which conazoles induced cytotoxicity. Adapted from Guan et al. 2020.

5.3.2. Fenpropimorph

Fenpropimorph {N-[3-(p-tert-butylphenyl)-2-methylpropyl] cis-2,6-dimethylmorpholine} is a morpholine fungicide that belongs to the Amines group, class II by the Fungicide Resistance Action Committee (FRAC) (FRAC, 2021) (Figure 5). Fenpropimorph is used in agriculture against fungal infections occurring on cereals, oleaginous, vine and banana crops (Marcireau et al., 1990). The amines fungicides are targeted to inhibit to a variable degree two target sites within the sterol biosynthetic pathway: the Δ14 reductase and the Δ8 → Δ7 isomerase.

Studies with fungal cultures applying morpholines cause the accumulation of sterol biosynthetic precursors such as 4,4-dimethyl-cholesta-8,14-dienol, ergosta-8,22-dienol(8), 4,4-dimethyl-cholesta-8,24-dienol, and ignosterol in *Saccharomyces cerevisiae* (Baloch et al., 1984). Finally, Gupta (2018) declared that Fenpropimorph is a mild irritant to rabbit skin and is associated with developmental toxicity and malformations in the same organism.

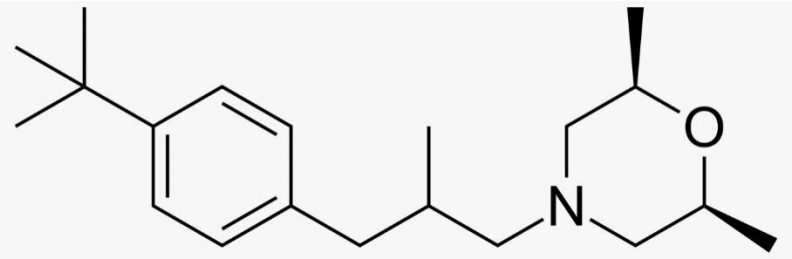

Figure 5. Graphical scheme of the amine fungicide: Fenpropimorph. Image available from Wikimedia Commons.

5.3.3. Mancozeb

Mancozeb (MZ) is formed by a mixture of manganese and zinc salts of the organosulfur compound ethylene-bis-di-thiocarbamate (EBDC) (Figure 6) (Scariot et al., 2016). MZ was first registered in the USA in 1948 as a broad-spectrum fungicide. Throughout its 70 years of use, MZ has been applied in industrial and agricultural applications standing out its agricultural antifungal properties for a variety of important crops (e.g., grapevine, tomato, potato, banana, and citrus), targeting nearly 400 different plant pathogens (Runkle et al., 2016). The increasing global consumption of fruits and vegetables, low purchase price, and continued non-selective fungicidal efficacy have made Mancozeb exhibit a faster production volume accounting for >20% of the global fungicide market in 2014 and a continuum forecast high production (Runkle et al., 2016).

Several studies have been performed on the model organism *Saccharomyces cerevisiae* under different conditions and concentrations of Mancozeb and many ECBDs (Kitagawa et al., 2003; Santos et al., 2009; Scariot et al., 2016). Specifically yeast adaptation to subinhibitory concentrations of MZ has been linked with protein degradation, oxidative response, and energy metabolism due to the high reactivity that MZ evokes in thiol groups of many proteins (Scariot et al., 2016). Furthermore, acute concentrations of MZ on aerobic exponentially growing *S. cerevisiae* strain BY4741, as well as deletion mutants, demonstrated that Mancozeb exhibits pro-oxidant activity on yeast, elevating mitochondrial Reactive Oxygen Species (ROS) and leading to a hyper-polarization of mitochondrial membranes and ultimately cause apoptosis.

The Environmental Protection Agency (EPA) study, initiated in 1977 and 1987, raised concerns about Mancozeb and other EBCDs because of particular health concerns, including

developmental and thyroid effects caused by the common degradation of ETU (EPA, 2005). Additionally, the World Health Organization (WHO) stated that the longer-term toxicity of MZ and ETU includes endocrine disruptive, mutagenic, carcinogenic and teratogenic risks (WHO, 1988). In 2012, several authors pointed out the genotoxic and premalignant changes in human ovarian and immune cells following exposure to MZ.

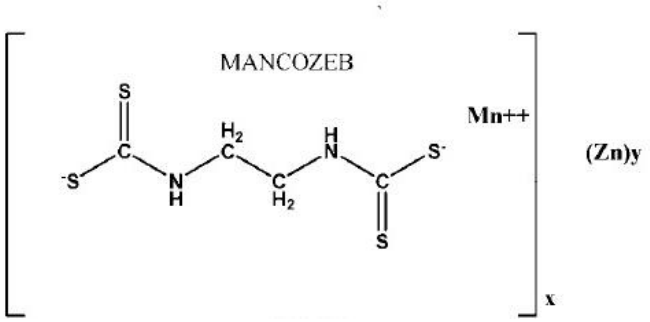

Figure 6. Graphical representation of the fungicide Mancozeb. Image available from Wikimedia Commons.

5.3.4. Pyrimethanil

Pyrimethanil (PYR; 4,6-dimethyl-N-phenyl-2-pyrimidamine) belongs to anilino-pyrimidines fungicides frequently used in vineyards, fruits, vegetables, and ornamentals mostly against the grey mold *Botrytis cinerea*. (Figure 7) (Müller et al., 2012; Gil et al., 2015; Lewis et al., 2016). Pyrimethanil's mode of action does not wholly decipher but researchers stated that PYR mainly inhibits the methionine biosynthesis and secretion of hydrolases essential for infection in many pathogenic fungi (Fritz et al., 2003; Milling and Richardson, 1995). Nonetheless, the exact primary target sites and resistance mechanisms are still not completely clear (Kanetis et al., 2008; Walker et al., 2013).

Hurlety (1998) studied Pyrimethanil in rodents. He concluded that it produces thyroid follicular cell tumors in rats and enhances hepatic thyroid hormone metabolism, possibly causing thyroid tumorigenesis. Therefore, enhancement of hepatic thyroid hormone metabolism and excretion are considered the mode of action of tumorigenesis (Waechter et al., 2010).

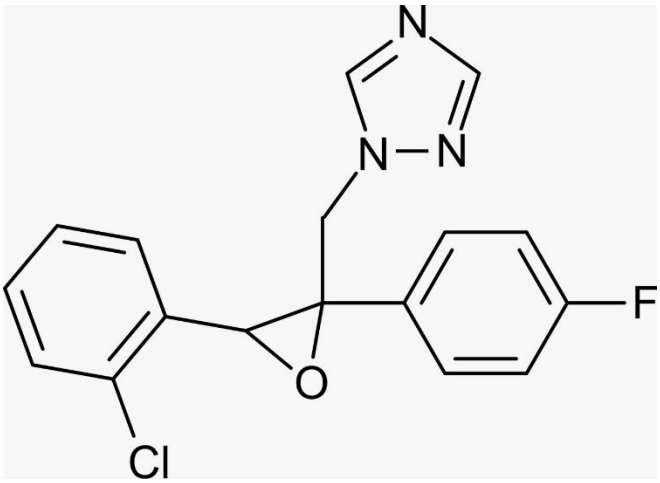

Figure 7. Graphical representation of the fungicide Pyrimethanil. Image available from Wikimedia Commons

5.4. Ecuador and banana fungicides

In Ecuador, the cultivation of Musaceae species represents critical support for the local economy and the nation's food security. According to the National Institute of Statistics and Census (INEC) (2011), there are 230 000 hectares of *Musa* spp plantations which are mainly distributed in three coastal provinces, Guayas, Los Ríos, and El Oro, representing the 92% and the 8% left are distributed in seven more provinces. Despite the country's economic and nourishing importance of the Musaceae species, its production is constantly threatened by several phytosanitary diseases (INIAP, 2021). Black Sigatoka, Panama Wilt, nematodes, insects, and viruses are the primary phytosanitary diseases of bananas and plantains reported in Ecuador. Most of the problems mentioned above have been managed or are managed using chemical pesticides as a safeguard measure to maintain the economy of all levels of banana and plantain producers and the local and international food supply of *Musa* spp.

Riveros & Arciniegas (2003) reported the growing virulence and dissemination of Black Sigatoka Streak Disease (BLSD) in all Latin American countries and most Caribbean islands, posing a severe threat to this crop. In Ecuador, the management of BLSD is principally based on the use of chemical fungicides applied in solo or cocktail mixtures. INIAP (2021) reported that the main groups of fungicides used to protect *Musa* spp plantations are systemic (which display multi-site action against the pathogen) and protectants (which display a

specific-site action against the pathogen) (Martinez et al., 2011; Santillan et al., 2017). Triazoles, Morpholines, Strobilurins, Carboxamides, and Guanidines belong to Systemic fungicides; Chlorothalonil and Mancozeb display protectans characteristics. The fungicides mentioned above have been widely used and commercialized in Ecuador under several names or formulations, as depicted in Table 1.

Many studies evaluate commercial fungicides' effectiveness and application costs against Black Sigatoka Streak Disease. Amines, Anilinopyrimidine, Dithiocarbamates, and Triazoles, comprise the most popular groups of fungicides evaluated in the field (Sabando, 2015; Franco, 2016; Portilla, 2017). Regarding Amines, Franco (2016) performed a study to evaluate the effect of amines fungicides against Black Sigatoka. He assayed IMPULSE®, VOLLEY®, and SEEKER®, three commercial amines fungicides widely used in Ecuador in Musa spp plantations. He found that VOLLEY®, Fenpropimorph as an active ingredient, showed prolonged and consistent efficacy after a trial of 49 days on the banana leaf and the lowest application cost.

On the other hand, Triazoles have been used all over banana-producing regions to control BLSD due to their high efficacy against the fungus and its accessible application costs. Sabando (2015) has pointed out the efficacy of triazoles for controlling BLSD in a banana-producing plantation in Quevedo, Los Rios province. Specifically, she found that OPAL®, a commercial triazole fungicide with epoxiconazole as an active ingredient, displayed the most efficacy and represented the lowest acquisition budget. Undoubtedly, the well-known efficacy of Triazoles fungicides for controlling BS; however, their application must be extremely careful because their excessive use leads to high selection pressure on *M. fijiensis*. Thus, the Fungicide Resistance Action Committee (FRAC) recommended including them in a rotation program with other groups of fungicides to lower the probability of generating new resistant strains of *M. fijiensis*. (FRAC, 2018). Calle (2008) summed up that BLSD should be controlled differently for the rainy season (December-May) by applying protectant or systemic fungicides in suspension or emulsion and also forming cocktails; meanwhile, for the dry season (June-November), the use of protectant with water or oil should be followed. The National Autonomous Institute of Agricultural Research (INIAP) suggested performing the abovementioned measures with a turbocharged motorized backpack pump three times a week. Nevertheless, this activity has been confined only to small farmers that usually possess a maximum of ten hectares of this staple. Big corporations that own the vast majority of hectares cultivated with bananas use aerial fumigations as the principal method to apply fungicides against Black Sigatoka infection.

The most practical way of controlling the leaf spot disease in large plantations is aerial spraying fungicides (Matthwes, 2009) (Figure 8). Aerial fungicide spraying is performed using a technique called 'Spray Off' that integrates several technologies, such as hydraulic nozzles or rotary atomizers with GPS, allowing the product to be sprayed automatically and to fall precisely in the right areas of the plantation. This technique guarantees that the fungicides reach the youngest unfurled leaves shielded from sprays applied from the ground by the mature leaves. The sprays usually consist of contact fungicides, Mancozeb and Chlorothalonil, and systemic fungicides such as Propiconazole, Trifloxystrobin, and Tebuconazole. Additionally, refined mineral oil is added to the spray to improve rain fastness and distribution across the leaf surface.

According to Harari and co-workers (2008), Tilt®, Calixin®, and Mancozeb and organophosphate fungicides are the most commonly used in aerial spraying. Sprays are often applied at 7-14 days intervals depending on the weather conditions, so managing meteorological information helps reduce the number of applications (Matthews, 2009). Martillo and Solano (2003) reported that the application cycles vary in the three major-banana-producer-provinces. For instance: Los Ríos presented more cycles of application which were between 25-29 cycles/ha/year; Guayas from 20-24 and El Oro from 12-16. Nonetheless, since 2000, the application cycles in Los Ríos reduced to 15 while El Oro increased, reaching an average of 18-23 cycles/ha/year. On the other hand, the cost of the application cycles, which used to be around $800 for Los Ríos, $600 for Guayas and $350 for El Oro, now they had been drastically reduced between $430-$800 in Los Ríos, $400-$600 in Guayas and $500-$600 in El Oro (Martillo & Solano, 2003). Finally, the extension of aerial spraying lasts an average of 26 weeks a year (Harari et al., 2008).

Figure 8. A typical aerial fungicide spray performed over a *Musa* spp plantation in Ecuador. (Ministerio de Comercio Exterior, 2017)

Table 1. Fungicides used against Black Sigatoka Streak Disease (BLSD) in Ecuador. (Adapted from Chong, 2016).

Chemical Class		Active Ingredient	Trade Name*	Description	Concentration **	References
Contact Fungicides	Benzene derivatives	Chlorothalonil	BRAVO 720, Bronco, DACONIL 720 SC	Interferes with the glutathione pathway, a coenzyme and 2-mercaptoethanol reducing thiol-group-based metabolism in the cell.	720 g/L	Rivera 2015 Chong 2016 Santillan 2017
	Dithiocarbamates	Mancozeb Metiram	Diethane 600 Polyran DF	Mancozeb is placed in the subclass of carbamate pesticides called dithiocarbamates. As a cholinesterase inhibitor, it affects the nervous system.	600 g/L 700 g/L	Matthews 2009 Alcivar 2014 Rivera 2015 Chong 2016 Santillan 2017 Ramón 2017 Galarza 2021
		Bitertanol	BAYCOR 500		500 g/L	Rivera 2015 Chong 2016
		Difenoconazole	Score, SICO 250	Preventive and curative. It	250 g/L	Chong 2016 Santillan 2017

Systemic Fungicides	Demethylation Inhibitors (DMI)		EC	penetrates the internal tissues of the plant avoiding being exposed to rain or environmental agents.		Sabando 2015
		Epoxiconazole	Opus OPAL 7.5 EC	It mixes easily with mineral oil; it can also be used in oil-water emulsions.	75 g/L	Chong 2016 Santillan 2017 Sabando 2015 Portilla 2017 Solorzano 2013
		Fenbuconazole	Indar 50 Of Indar 2 Of	An emulsion (oil in water) fungicide.	50% P/V 250 g/L	Rivera 2015 Chong 2016
		Myclobutanil	Rally 40 WP Sisthane	Protective and curative properties against a wide range of fungi. It protects new shoots and controls fungus for up to 4 days after infection begins.	400 g/Kg	Rivera 2015 Chong 2016
		Propiconazole	Tilt 250 Bumper	It acts inside the plant controlling the pathogen during the formation of the first haustorium	250 g/L	Rivera 2015 Chong 2016
		Tebuconazole	SILVACUR Combi 300 EC	Concentrated emulsion. Inhibitor of ergosterol biosynthesis and germ tube destroyer. Apply it alone or in a mixture in association with agricultural oil.	225 g/L Tebuconazole + 75 g/L Triadimenol	Rivera 2015 Sabando 2015 Chong 2016 Santillan 2017
		Tetraconazole	Eminent 100 Ce	Concentrated emulsion for foliar spray treatment. Its treatment is recommended preventively and sequentially.	100 g/L	Rivera 2015 Chong 2016

		Triadimenol	Bulldock 25 Sc, Caporal		250 g/L	Rivera 2015 Chong 2016
	Amines	Spiroxamine	IMPULSE	Emulsifiable concentrate. It must be mixed at the product's recommended dose in 12/14 l of agricultural oil. Emulsion mixtures can also be made using water oil.	800 g/L	Rivera 2015 Chong 2016 Franco 2016 Santillan 2017
		Fenpropimorph	VOLLEY® 88 OL	Oil-miscible liquid. It is mixed only with agricultural oil.	880 g/L	Rivera 2015 Chong 2016 Franco 2016 Santillan 2017
		Fenpropidin	SEEKER	Emulsifiable concentrate for emulsion or suspension preparations. It should be applied preventively.	750 g/L	Rivera 2015 Chong 2016 Franco 2016 Santillan 2017
		Tridemorph	Calixin, Musaclean, Tribanex, Finder, Calined	It is used preventively and has curative activity, effectively combating BLSD in banana and plantain crops.	860 g/L	Rivera 2015 Chong 2016
	Qo Inhibitors (QoI)	Azoxystrobin	BANKIT	Concentrated suspension. Only for prevention methods.	250 g/L	Rivera 2015 Chong 2016
		Pyraclostrobin	COMET GOLD	Emulsifiable concentrate containing pyraclostrobin (inhibits mitochondrial respiration) and Fenpropimorph inhibit the biosynthesis of ergosterol.	100 g/L Pyraclostrobin + 375g/L Fenpropimorph	Rivera 2015 Chong 2016 Santillan 2017
		Trifloxystrobin	TEGA 500	Concentrated suspension. Apply it in a tank mixture with a	500 g/L	Rivera 2015 Chong 2016 Santillan 2017

				product with a different mechanism of action like Siganex 600 SC.		
	Anilinopyrimidines (AP)	Pyrimethanil	SIGANEX 600 SC	Concentrated suspension. Apply it alone or in a mixture in association with agricultural oil at the dose	600 g/L	Rivera 2015 Chong 2016 Santillan 2017 Portilla 2017
	Benzimidalos	Benomyl	Benomyl 50 Pm Benomyl 50 Wp	It is a broad-spectrum fungicide that controls a large number of diseases fungi in horticultural crops, ornamental crops, cereals, fruit trees, vineyards and grapevines.	500 g/L	Rivera 2015 Chong 2016
		Carbendazim	Carbedazaim 500 Sc Carbenpac 500 Sc	It is a broad-spectrum systemic fungicide with preventive and curative action.	500 g/L	Rivera 2015 Chong 2016
		Thiophanate	Nucilate 50 Sc, Thiofin 50 Sc, Topsin M	Protective and curative action, when applied to the soil, is absorbed by the roots of the plants and transferred to the foliage via the xylem and phloem.	500 g/L	Rivera 2015 Chong 2016
		Thiabendazole	Mertect 20	It controls the fungal complex of crown, finger and neck rot in banana and plantain crops.	200 g/L	Rivera 2015 Chong 2016
		Thiophanate-methyl	ELITE, Tiofanato Metílico, Topsin M	Preventive-curative systemic fungicide. It has a broad spectrum of action.	700 g/L	Rivera 2015 Chong 2016

	N-phenylcarbamates	Diethofencarb	POWMYL	Concentrated suspension. Preventive and curative action, belonging to the group of N-phenyl carbamates		Chong 2016
	SDHI	Boscalid	CUMORA SC CANTUS Wg	Concentrated suspension; a dose of 0.4 liters per hectare. Usually, mixed with VOLLEY	500 g/L	Rivera 2015 Chong 2016 Santillan 2017
		Fluopyram	VERANGO		500 g/L	Rivera 2015 Chong 2016
		Isopyrazam	REFLECT SUNJET	Emulsifiable concentrate or emulsion or suspension preparations. Inhibitor of complex II in the respiration process of the pathogen. It should be applied preventively.	125 g/L	Rivera 2015 Chong 2016 Santillan 2017
	Guanidines	Dodine	SYLLIT 400 SC POWDINE	Curative and protective action. Monosite inhibitor acts on the cell membranes of fungi. It's recommended to make the application in water/oil emulsion, using between 5 and 7 L of mineral oil per hectare.	400 g/L	Rivera 2015 Chong 2016 Santillan 2017

*Some trade names also include mixes with other active ingredients.

** Concentration of active ingredient per liter of commercial product according to manufacturers.

5.5. Banana fungicides and health in Ecuador

Ecuador health centers face a challenging duty to maintain a record of the people affected by fungicide poisoning due to many cases being treated at home, going unnoticed, or being difficult to trace (Mollocana, 2020). Pesticide poisoning presents a high risk for workers or populations that live near banana plantations. Regarding banana workers, they are directly affected by pesticide (fungicide) exposure due to improper exposure of personal protective equipment (PPE) such as gloves and facemasks, contact with chlorpyrifos-impregnated in bunch covers, contact with residual water contaminated with fungicides residues and aerial sprays (Brisbois, 2016; Harari et al., 2008). Meanwhile, people living near or inside banana crops are exposed to fungicide by aerial spraying to combat BLSD.

Pesticide management and its repercussions on people exposed for a long time is an underrated problem in Ecuador due to scarce literature and few experiments (Mollocana and Gonzales, 2020). Nevertheless, there are some reports that discuss the pesticide effects on people that worked or lived near potatoes, flowers and banana plantations (Orozco et al., 2009). In a study carried out by Brisbois (2016), he argued that several health conditions of banana workers might be associated with continuous and uncontrolled aerial fungicide sprays performed by banana corporations in El Oro province. This perspective is corroborated by a study that used the fluorescent tracer technique following a typical aerial spray procedure to demonstrate the presence of fungicides from aerial spraying in workers and people that live near commercial banana plantations (Harari et al., 2008). In this study, fungicide exposure originated from the drift of the aerial sprays that reached workers in their workplaces (even though they are using PPE), outside-plantation houses with residents and even a local school. Furthermore, it claimed that banana workers do not get prevented by the schedule of these sprays. Many times, they performed their work activities or other regular activities (like eating) while the aerial spraying was occurring above them. Surprisingly, many statistics report that communities near the commercial *Musa* spp plantations cannot access clean and safe water supply because options are limited. Thus, villagers sometimes obtain their water in tanks from supply trucks that visit the area and keep it in open containers near their houses. Or sometimes, they use the water that flows on the banks of the banana plantations for washing clothes, bathing and cooking.

National Federation of Agroindustrial Workers, Peasants and Free Indigenous People of Ecuador (FENACLE) conducted a study on 247 former employees of banana companies

that used Dibromochloropropane (1,2-dibromo-3-chloropropane) (DBCP) during 1979 to 1984. As a result, FENACLE found four cases of male reproductive disorders (azoospermia: total loss of spermatozoids in semen), which they considered relatable to high exposition to DBCP; this corroborated the other reports presented by Centro American organizations against this chemical used widely in Central America commercial banana plantations controlled by the United Fruit Company (Harari et al., 2008).

5.6. Resistance

Fungicide resistance is a heritable and stable trait that reduces the fungicide sensitivity of a particular fungus (McGrath, 2004). Fungus obtained resistance through an evolution process. Therefore, fungicides with a single site of action (systemic) present a high risk for resistance development compared to fungicides with multiple sites of action (protectant). Most fungicides developed today have a single mode of action because this type of action is associated with a low potential for negative impacts on the environment.

A significant concern for designing new fungicides is the threat of fungicide resistance. In that sense, the fungicide industry has focused and conducted research concerning fungicides' mode of action, sensitivity in the field, and resistance risk. Consequently, the use of strategies minimizes both the accumulation of fungicide resistance and the loss of efficacy of current fungicide classes.

Fungicide resistance and cross-resistance have grouped manufacturers into the Fungicide Resistance Action Committee (FRAC). FRAC is an international organization that ensures the correct management of fungicide resistance in all available fungicide formulations. FRAC has produced several resources on various aspects of fungicide resistance by grouping the fungicides based on several criteria easing the understanding of the resistance risk of the different fungicide groups. Nevertheless, these assessments of the resistance risk have become difficult due to the unpredictability of cross-resistance, which sometimes does not follow the described mode of action of the product, and these associations can become pretty complex.

FRAC has created the Banana Working Group (BWG), which aims to represent all significant banana-growing regions globally and where discussions and solutions about pathogen resistance in *Musa* spp are treated. Besides, the working group develops fungicide resistance strategies in banana cropping (FRAC, 2022). Furthermore, BWG has delineated some strategies for fungicide groups directly used against BLSD.

FRAC has registered some cross-resistance in Demethylation Inhibitors (DMIs), where Epoxiconazole is included. Therefore, the FRAC's recommendations to Banana plantations for controlling Black Sigatoka worldwide stated that DMIs could be applied in mixtures of two or more DMIs to provide good biological efficacy. Nonetheless, they do not provide an anti-resistance strategy and must be treated as a solo DMI for resistance management. Therefore, FRAC (2018) recommended the following guidelines:

- Demethylation Inhibitors are recommended to use in mixtures with non-cross resistant modes of action, mixing all at manufacturers' recommended effective rates.
- Demethylation Inhibitors fungicides are performed better for alternation **(if possible, two cycles with other MoA in between)** and total label rate with other, non-cross resistant modes of action.
- Apply a maximum of 8 DMI fungicides applications, but not more than 50% of the total number of sprays.

Amines (Fenpropimorh) have a narrower spectrum of activity than the DeMethylation Inhibitors (DMI). Therefore, they can be used as a sole treatment but are often used in mixtures with DMI's to control powdery mildews and rusts. In 2018, the Banana working group, a FRAC's working group, elaborated guidance where they stated the applications of Amines fungicides controlling Black Sigatoka should follow the following:

- Amine fungicides can be used with a maximum of 2 consecutive sprays (under a block treatment).
- Amines should be alternated with another fungicide with a non-cross-resistant mode of action.
- Amines do not exceed the maximum of 15 applications containing but not more than 50% of the total number of sprays. (FRAC, 2018).

In Ecuador, MAATE, MAGAP, and MSP have made significant efforts to control pesticides and proper environmental management during banana production processes and protect the environment and the health of residents of producing areas (Ministerio de Comercio Exterior, 2017).

6. Black Sigatoka Streak Disease

6.1. History of Black Sigatoka Streak Disease

The fungus of Black Sigatoka *Mycosphaerella fijiensis* Morelet was first described in Viti Levu belonging to Fijian Islands in 1963 (Crous et al., 2011; Ploetz et al., 2003) but probably was widespread in the Southeast Asian/South Pacific region by that time (Ploetz et al., 2003). Several islands presented the disease: American Samoa, the Cook Islands, French Polynesia, Micronesia, Papua New Guinea, Tonga, Fortuna Islands, the Solomon Islands, and Samoa (Promusa, 2020).

In Asia, Stover (1978) suggested that BLS might have affected Taiwan banana plantations as early as 1927. The disease has been found in China, Butan, Malaysia, Indonesia, Philippines, Thailand, Singapore, and Vietnam. On the other hand, Gabon was the first African country to present an authenticated BLS report in 1980. Benin, Cameroon, Congo, Nigeria, Ghana, and Togo are other West African countries presenting Black Sigatoka.

Black Sigatoka was reported in Honduras in the Americas by 1972 (Ploetz 2003). Then, it spread to all the regions following this order: Belize (1975); Guatemala (1977); Nicaragua, El Salvador, and Costa Rica (1979); Mexico (1980); Panama and Colombia (1981); Ecuador (1986); Venezuela (1991); Peru (1994); Bolivia (1996); Brazil and Florida in the USA (1998); Bahamas (2004); French Guiana (2008); and finally most Caribbean islands (Figure 9).

Figure 9. Chronological spread of Black Sigatoka Streak Disease in the banana-growing regions of the Americas (Promusa, 2020).

6.1.1. History of Black Sigatoka in Ecuador

In 2003, Ecuador possessed approximately 150.000 hectares of banana plantations in the Coastal Region infected by Black Sigatoka. This number was constituted mainly by three provinces Los Ríos (45.000 ha), El Oro (44.000), and Guayas (43.000). Historically, Los Ríos and El Oro always had the highest and lowest infection rates. Nevertheless, this situation changed dramatically in 1998 when the 'El Niño' phenomena severely struck the coastal profile of Ecuador (Martillo & Solano, 2003). This incident radically changed Black Sigatoka's behavior in Ecuador, ferociously affecting more provinces. Therefore, all the BSLD's official and non-official controlling programs failed with severe economic consequences for the farmers, stakeholders, and consumers. Fortunately, this environmental disaster called to action governmental institutions and private initiatives for searching the causes that provoked the failure to control BS with chemical fungicides.

6.2. General description

Black Sigatoka or Black leaf streak disease (BLSD) is a leaf spot disease caused by the fungus *Pseudocercospora fijiensis* (Morelet) Deighton (teleomorph *Mycosphaerella fijiensis* Morelet) (Fullerton, 2019). The fungus {*Mycosphaerella fijiensis* Morelet} attacks several species of Musaceae family-like *Musa acuminata, Musa balbsiana,* and their interspecific hybrids. *M. fijiensis* colonizes the surface of banana and plantain leaves by rain-splashed and wind-borne conidia and airborne ascospores; then, it produces the partial or entire necrosis of leaves (Figure 10). Thus, the plant reduces its photosynthetic foliage area leading to a reduction in product yield depending on the extent or severity of the disease (Etebu, 2011).

Nowadays, Black Sigatoka constitutes the dominant leaf spot disease. BS has replaced other important leaf spot diseases such as Yellow Sigatoka (*Mycospharella musicola* leach), and now BS has spread all over the tropics worldwide. This disease can cause yield losses up to 50% during the plant crop cycle (Stover, 1983; Pasberg-Gauhl, 1989; Mobambo et al., 1993). Therefore, its widespread distribution and destructive infection threaten the economy of thousands of millions of farmers that cultivate the crop (Etebu, 2011). Furthermore,

control measures account for 15-27% of the total annual production costs (Stover, 1980a, b; Stover and Simmonds, 1987; Etebu, 2011).

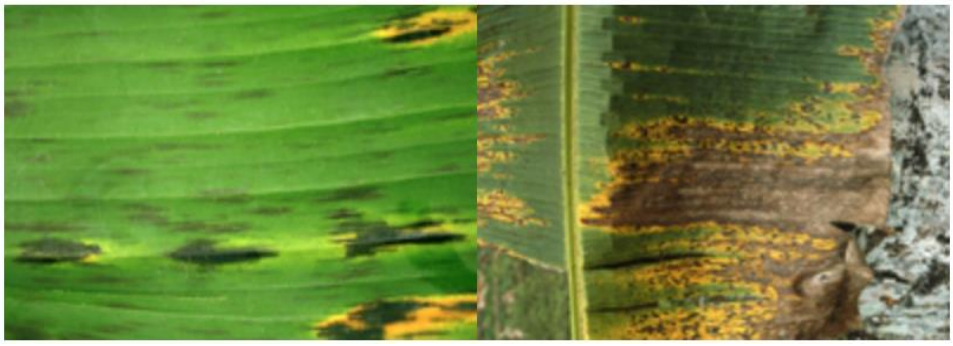

Figure 10. First symptoms of Black Sigatoka Streak Disease on the leaf surface of a *Musa* spp. *Left side:* The first symptoms of black Sigatoka disease are tiny, chlorotic spots that appear on a loose leaf's bottom (abaxial) surface. *Right side:* The spots grow into thin brown streaks limited by leaf veins. The streak's color gets darker and is visible on the top (adaxial) surface. Then, lesions enlarge, adopting a fusiform or elliptical form, and darken to give the particular black streaking of the leaves. (Source Promusa 2020).

6.3. Pathogen Biology

6.3.1. Asexual reproduction

Pseudocercospora fijiensis is the asexual form or anamorph of the fungus. *P. fijiensis* possess conidiophores that carry conidia which are born singly and terminally. These conidia are smooth, long, and have three or more septa (Figure 3). Also, they are pale to a light olive-brown (Bennett, 2003).

Humidity is an essential factor for conidia germination. Periods of high relative humidity (92-100% relative humidity) are convenient for conidia to infect the leaf through a stoma. If the humid conditions persist, hyphae can emerge from the stomata, grow along the leaf surface and penetrate through other stomata, thus enlarging the lesions. Conidiophores emerge through the stomata as compact masses of mycelium. It is important to note that stromata may also develop in young spermatogonia.

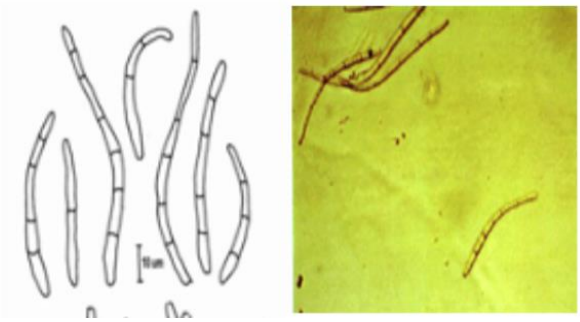

Figure 11. *Pseudocercospora fijiensis* conidia description. Left side, conidia are borne on the conidiophore singly and terminally. Right side, the spores are pale to a light olive-brown and are smooth, long, and have three or more septa. (Promusa, 2020).

6.3.2. Sexual reproduction

Mycosphaerella fijiensis is the name given to the sexual form or teleomorph of the pathogen. To produce the sexual form, *M. fijiensis* first develops many spermagonia on the lower surface of the leaf as the lesions collapse. Spermagonium (Figure 12) is dark and pearlike in shape. In humid conditions, these structures may ooze large quantities of spermatia (the male reproductive cells). Spermatia are small and cylindrical cells that fertilize trichogynes (female receptive hyphae) (Bennett, 2003).

Pseudothecia are formed within the mature lesions after fertilization is complete. Asci, sac-like structures, have two cell walls (bitunicate) and contain eight sexual spores (ascospores) that are lined up two-by-two. Pseudothecium does not have pseudoparaphyses (sterile elements) (Bennett, 2003). Ascospores have one septum, and they are colorless. One spore cell may be slightly broader than the other cell, and the spore may be slightly constricted at the septum.

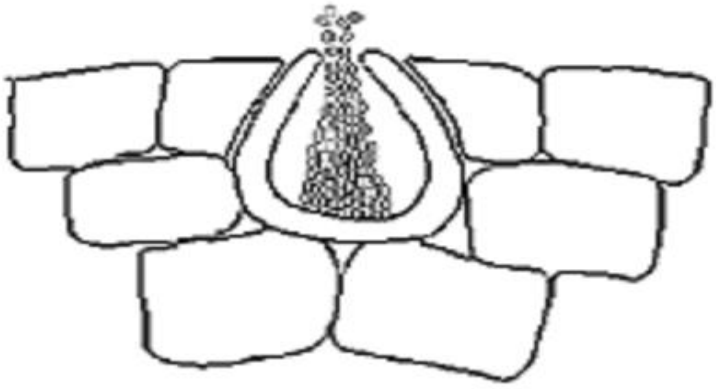

Figure 12. Spermagonium of Mycosphaerella fijiensis. Spermagonium is dark with a pearlike shape. In humid conditions, spermatogonium may ooze large quantities of the male reproductive cells (spermatia) that fertilize neighboring female receptive hyphae (trichogynes).

6.4. Life cycle and Epidemiology

Black Sigatoka infection occurs primarily via the stomata on young, unfurling leaves (Hernandez et al., 2006; Fullerton et al., 2019). BS utilizes ascospores and conidia as propagules for dispersion. Both ascospores and conidia need high humidity to spread the disease on a plantation. In the case of conidia, these asexual spores disperse during rain-wash and splashing, causing the local spread of the disease; researchers have found conidia to remain viable for up to 60 days on leaf surfaces but only 18 days on the skin of the fruit (Hanada et al. 2002). Meanwhile, ascospores constitute the primary means of long-distance dispersal, and their spreading mainly occurs during extended periods of moist conditions.

M. fijiensis does not form extensive amounts of conidia; therefore, ascospores are more critical in the disease cycle (Bennett, 2003) (Figure 13). Although the disease is highly destructive to leaves, evidence of fruit infection is rare.

P. fijiensis has a relatively long incubation period. Its first symptoms resemble tiny chlorotic flecks that appear on the abaxial side of the leaf 10 to 14 days after infection but often do not progress to rusty streaks for over 20 days after infection, even on highly susceptible 'Cavendish' types (Meredith and Lawrence 1969; Meredith et al. 1973; Jones 2000). Researchers first described BLSD into six stages in 1969 (Meredith and Lawrence, 1969). However, later in 1987, Eric Fouré added some more features to their description, and its

characterization is the current standard for identifying the pathogen infection in banana plantations (Figure 14).

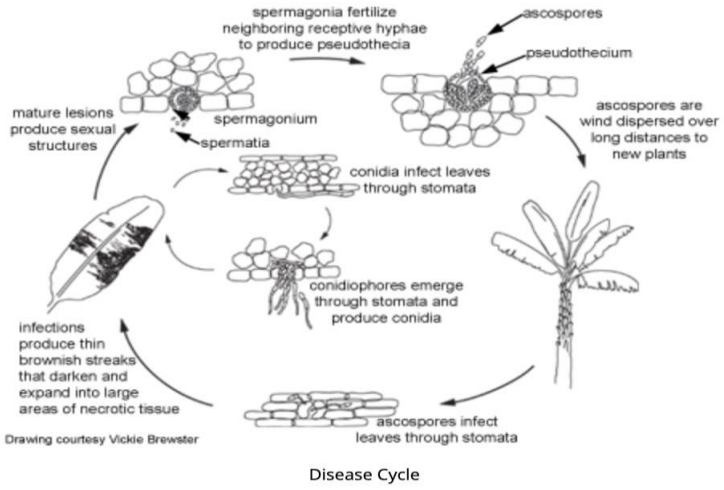

Figure 13. The life cycle of *M. fijiensis* Morelet. (Bennett et al., 2003).

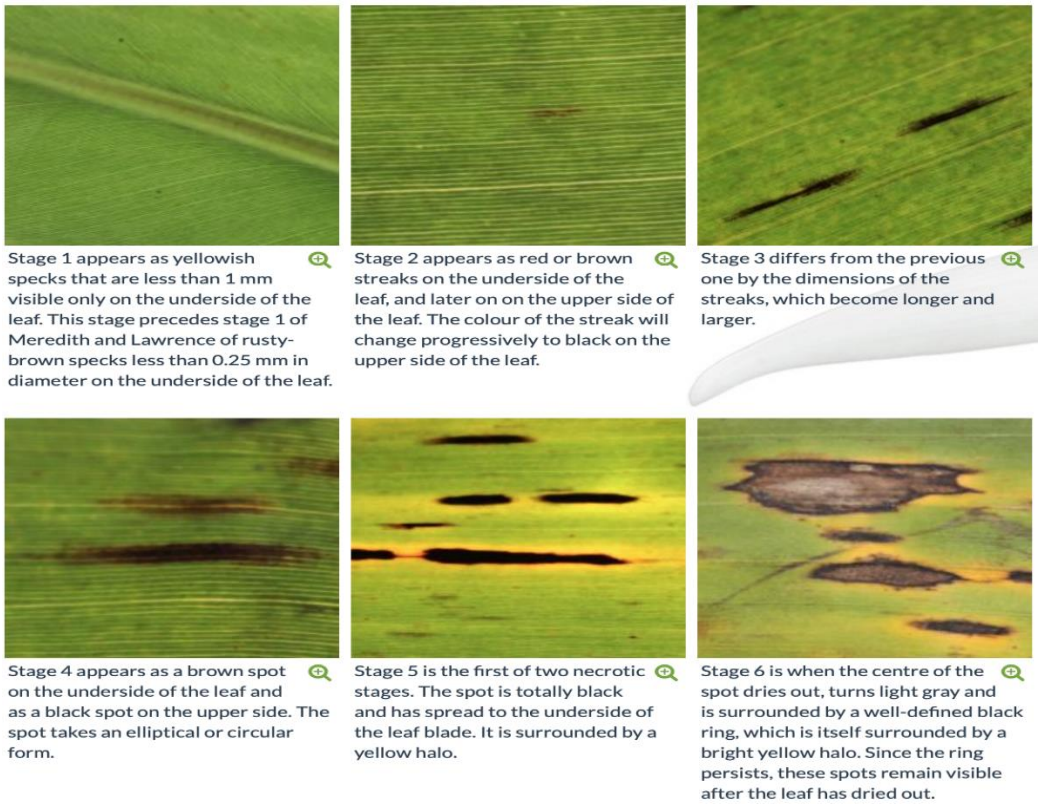

Figure 14. Six stages of Black Sigatoka Streak Disease (Promusa, 2020).

6.5. Principal methods to control BLSD

Controlling Black Sigatoka disease requires the implementation of an organized and well-structured Integrated Pest Management (IPM). This plan results from combining several other strategies such as biological, chemical, cultural, and physical methods that guarantee the minimization of economic, health, and environmental risks (Orozco-Santos et al., 2019; Nelson, 2008).

To implement an efficient integrated management plan for controlling the Black Sigatoka streak is necessary to gather precise and current information about the cultivar (plant age, disease susceptibility, pathogen interaction, and phenology), the pathogen (fungal species, type of reproduction, genetic structure, spread, source of inoculum, survival, incubation period, and disease cycle), and the weather (amount and distribution of precipitation, temperature, dew, solar radiation, cloud cover, and relative humidity) (Orozco-Santos & Orozco-Romero, 2006) (Figure 15). Finally, the factors mentioned above are specific for each geographical region that *Muse* spp individuals are cultivated. Therefore, continuum studies are required to elaborate agricultural practices that meet the necessities of a particular climate, cultivar, and pathogen conditions from a determinate banana-growing region.

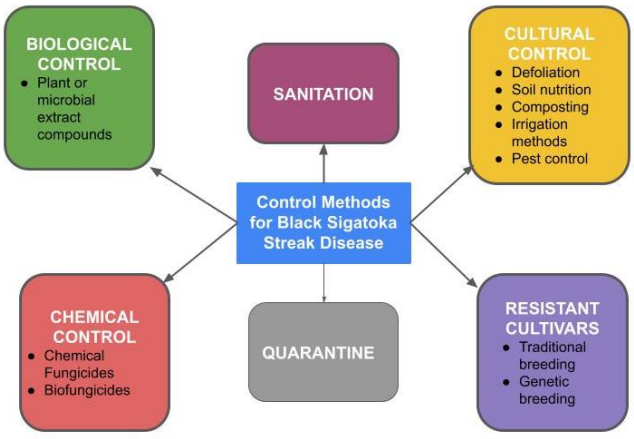

Figure 15. Types of control applications for controlling Black Sigatoka Leaf Streak. (Orozco-Santos & Orozco-Romero, 2006).

6.5.1. Biological control

Biological control (biocontrol) uses microorganisms and animal/plant compounds to generate agents for eradicating or controlling Phyto/zoo diseases. Indeed, most strategies proposed to control BLSD in many countries worldwide came from plant extracts. For example, Paola (2006) used extracts from *Momordica charantia* and *Senna reticulata*. As a result, she significantly reduced the incidence and severity of BLSD on plants infected artificially with *M. fijiensis* conidia. Likewise, Dooh and collaborators (2021) experimented with seed extracts from *Thevetia peruviana* (Pers.) that significantly reduced *M. fijiensis* germ tube growth and inhibited approximately 60-90% and 40-80%, respectively. Furthermore, Seydou et al. (2021) performed in vitro and in vivo tests with twenty Ivonian flora extracts that showed biopesticide properties against *M. fijiensis* conidia/germ tube development. Thus, this plant approach had revealed some interesting features from plant extracts that Riveros et al. (2003) and Polanco (2004) had attributed to the presence of a variety of secondary metabolites such as flavonoids, coumarins, polyphenols, saponins, triterpenes that are known to act as antifungal compounds against *M. fijiensis*.

Nonetheless, Marin and co-workers (2003) stated that despite the promised biocontrol activity that many plant extracts performed, no biological control methods had been adopted in commercial plantations. This might be due to the nature of the earlier reviewed methodologies, which are effective against BLSD development; however, these treatments correspond more to a preventive treatment than a curative solution. Therefore, to protect banana and plantain tree varieties against *M. fijiensis*, types of treatments involving synthetic pesticides and biopesticides are necessary to eradicate the pathogen or at least control it effectively. Additionally, more studies on the novel plant must extract as potential bio-pesticides to lower the use of mainstream chemical solutions that carry out several risks. Therefore, the challenge of controlling this polycyclic fungus by biological methods is immense.

6.5.2. Cultural management

Cultural management is one of the critical control strategies to avoid or minimize BS infection on banana/plantain cultivars. The main cultural management strategies are cutoff of leaves, irrigation methods, soil nutrition, and mini composting. So, the objective is to delete any trace of inoculum, difficulting its growth and spreading, creating more healthy cultivars.

The total or partial elimination of infected leaves is the most critical measure to reduce or eradicate the fungus inoculum (Orozco-Santos et al., 2008). The total cuts of leaves occur if the infection has passed the 40% of the leaf surface, while partial cuts happen when the leaves present infection rates lower than 30-40% of the leaf surface. According to Gauhl (1994), sick leaves on the plant present the most extended production period and release of the pathogen's spores for twenty weeks. Furthermore, although in the soil, leaves decompose after ten weeks, the leaf lesions loaded with B.S. can continuously discharge spores for almost 30 days after the cutting (Villalta & Guzmán, 2005). Therefore, cut leaves should be orderly disposed of in the soil followed by urea application, guaranteeing faster degradation of the organic matter and contributing as a fertilizer for the plantations avoiding future dispersal after the dissection. Notably, soil nutrition and drainage strategies also contribute to maintaining low levels of Black Sigatoka infection.

6.5.3. Chemical Methods

Systemic and contact fungicides represent an efficient and popular control for Black Sigatoka (Foure et al., 1983; Quevedo et al., 2018) **(**Table 2). Thus, control programs in almost all banana-growing regions were primarily based on the protectant fungicides mancozeb, usually applied in water or in combination with oil and chlorothalonil (Bennett et al., 2003).

Before Black Sigatoka landed in banana plantations in Ecuador, INIAP announced in 1986 some chemical measures other countries took to control BSLD. Thus, they reported using fungicide cocktails by aerial means to control BS. These cocktails consisted of two or more fungicides, especially mancozeb, benomyl, chlorothalonil, and tridemorph. The application of a plethora of contact and systemic fungicides by aerial and terrestrial means reached an average of 24 application cycles/year (Sanchez Valdivieso et al., 2021). It is important to note that applying more fungicides cycles per year in the country means more protection for the plantation.

Nonetheless, according to Cervates (2019), double aerial applications of commercial fungicides (Difenoconazole and Fenpropimorh) showed less chlorophyll content in the third leaf of commercial cultivars of *Muse* spp (genome AAA) in El Oro province. Therefore, this traditional outlook, more fungicide application equals better protection, has raised several problems such as pathogen resistance, health conditions, and environmental risks (Sanchez Valdivieso et al., 2021). So, the quest for better agents that do not cause resistance and side

effects both for human populations and the environment has generated the application of alternatives like bio-fungicides and organic fungicides (Chang, 2021).

Table 2. Description and examples of Contact and Systemic fungicides used against BS.

Type of fungicide	**Description**	**Examples**	**Reference**
Contact Fungicides	They only protect the parts of the leaf sprayed by spraying, and the spraying does not affect the already established infection. Also, it prevents spore germination.	Dithiocarbamates and Chlorothalonil	Orozco-Santos, 2008
Systemic Fungicides	They are used for their preventive and curative action. The fungicide is mobilized internally in the plant tissue. However, this fungicide group generates resistance due to active ingredients acting on specific sites of the fungal cells.	Benzimidazoles, Triazoles, Strobilurins and Morpholines.	Ayala et al., 2014

6.5.4. Resistant cultivars

There are banana cultivars that can resist Black Sigatoka Streak Disease. The Honduran Agricultural Research Foundation (IFHA) developed some important BLS banana-resistant species (Table 3). However, commercially essential types of bananas lack resistance (including export desert AAA, highland AAA, AAB plantain, and AAB dessert cultivars). Nevertheless, traditional breeding of *Muse* spp has several limitations regarding high levels of sterility, need for interploidy crosses, low seed germination rate, and long growing cycles (Sánchez & Santos, 2010). Furthermore, suppose a traditional breeding program achieves a resistant hybrid that generates high productivity and resistance to some biotic and abiotic stress. In that case, they often are susceptible to other diseases (Fusarium wilt disease), pests (burrowing nematodes: *Radopholus similis*), and critical commercial characteristics might be lost (shelf life and organoleptic properties of the pulp), leading to a potential lowering of demand of that hybrid by the market (Nelson, 2008; Sanchez & Santos, 2010). Thus, the use of genetic transformation

techniques has the potential to rise as a promising field to produce resistant individuals of bananas.

Genetic Engineering constitutes a novel approach to generating cisgenic bananas. Genetic transformation surpasses banana sterility using embryonic stem cell suspensions. Currently, there are two developed genetic transformation methods: the bombardment of particles and transformation mediated by Agrobacterium (Sanchez & Santos, 2010). The latter methodology is the most used due to the relatively low number of gene copies integrated into the plant genome. Plant breeders modify a particular set of genes using genetic transformation to obtain the desired characteristics; meanwhile, organoleptic or postharvest characteristics are maintained as the original cultivar. Furthermore, other approaches like overexpression of pathogenesis-related protein (PR) antifungal proteins and bacterial hydrolytic enzymes and utilization of RNAi-mediated gene silencing of a pathogenesis-related protein encoded by pathogens are currently used to generate resistance in *Musa* spp (Sowmya et al., 2016).

The Center of Biotechnological Research of Ecuador (CIBE) performed basic and applied research, searching for solutions and innovations for the agricultural sector in Ecuador. Among the CIBE's priorities are diagnosing and controlling pests and improving resistance to pathogens of several commercial crops, including *Muse* spp. For example, the first genetic transformation of bananas by Agrobacterium (Sanchez & Santos, 2010) and the identification of resistance genes (Saavedra & Santos, 2013) were developed under CIBE installations. Additionally, CIBE has conducted research to characterize the pathogen biology of *M. fijiensis* in Ecuador. Thus, the molecular characterization of the populations of *M. fijiensis*, the estimation of the sensitivity to fungicides (Triazoles, Strobilurins, Benzimidazoles, and Carboxamides) of *Mycosphaerella fijiensis* and the construction of a collection of isolates of *M. fijiensis* from all the banana-growing area represent some efforts to learn more about the causative agent of Black Sigatoka in Ecuador (CIBE, 2022).

Table 3. List of FHIA (Fundación Hondureña de Investigación Agrícola) resistant bananas to abiotic and biotic agents. Not all of these banana hybrids are available in Ecuador. Adapted from Nelson 2008. *BLS = black leaf streak; BN = burrowing nematode; CR = crown rot; FW = Fusarium wilt.

Resistant cultivar		Description*	Uses
Hybrid	**Genome**		
FHIA-01	AAAB	Highly resistant to BLS; resistant to Race 1 FW; tolerant to BN; Resistant to CR; tolerant to drought; tolerant to cold temperature; female parent is 'Dwarf Brazilian.'	Pome-type, apple flavor dessert banana; also green cooking
FHIA-02	AAAA	Highly resistant to BLS; resistant to CR	Sweet, similar to Cavendish
FHIA-03	AABB	Resistant to BLS; resistant to Race 1 FW; drought-resistant; highly vigorous; semi-dwarf; tolerant to marginal conditions; one parent is 'Bluggoe.'	Cooking banana, also for dessert
FHIA-17	AAAA	Resistant to Race 1 FW	Dessert banana Can be cooked
FHIA-18	AAAB	Resistant to BLS; long shelf life; few skin blemishes	Sweet acid (apple flavor) dessert banana
FHIA-23	AAAA	Tolerant to BLS; one parent is 'Highgate' (a 'Dwarf Bluefields')	Dessert

6.5.5. Quarantine and Sanitation

Quarantine measures are usually taken by governments or banana farmers when a banana-growing region has not yet been infected with Black Sigatoka (Bennett & Arneson, 2003). Proper quarantine and sanitation measures may protect against two standard means of long-distance inoculum dispersal—leaves and rhizomes (Bennet & Arneson, 2003). According to Espinosa (1986), workers of an infected banana cultivar should never visit unaffected banana plantations without changing their clothes and taking three weeks of quarantine. Besides, he recommended that all tools, utensils, and vehicles from an affected plantation must be washed and disinfected to avoid spreading the disease. It is noteworthy to mention that in developing countries, Bennett and Anerson (2003) have pointed out that farmers often use contaminated banana leaves to protect the fruit when bananas are transported by truck. Therefore, performing routine reconnaissance and inspections of infected and non-infected plantations is mandatory for sanitation and containment purposes.

7. Saccharomyces cerevisiae under fungicides treatment

7.1. Phenotypic responses

Most yeast-bioassays encountered in the literature exposed a yeast-growing population to sub-inhibitory fungicide concentrations to evaluate yeast's responsiveness (Gil, 2014; Scariot, 2016; Briz-Cid, 2020; Dias, 2010; Casalone, 2010). This approach is followed because these fungicide concentrations do not kill *S. cerevisiae* culture, and their metabolic response can be quantified by physiologic, genomic, proteomic, chemogenomic, and transcriptomic means. Usually, the 50% inhibition of yeast's growth rate (IC50) is the most common variable to test the fungicide effect on growth. Nonetheless, authors may consider IC20 exposure conditions to represent a measure of general cytotoxic concentration reported in other studies with different organisms. Also, many authors considered that IC20 provides transcriptional insights associated with moderated phenotypic effects avoiding a substantial cellular deterioration, thus representing a presumably transient cellular response (Yasokawa & Iwahashi, 2010; Seeland

et al., 2012; Gil et al., 2014). Other important considerations of this type of study are the use of active fungicide ingredients, yeast laboratory strains, time intervals, and culture media.

7.1.1. Apoptosis bioassays

Apoptosis refers to programmed cell death with an essential role in the development and homeostasis of metazoan organisms (Kerr et al., 1972; Wyllie et al., 1980; Madeo et al., 1997). It allows the rapid removal of damaged cells that could inflame the surrounding cells with their cytoplasmic contents. Conversely, necrosis is a form of cell death that results from overwhelming cellular injury; cells lyse and release cytoplasmic material (Madeo et al., 1997). In yeast, programmed cell death is performed by proteases called caspases. Yca1, the only identified metacaspase in yeast, plays a crucial role in regulating apoptosis in *S. cerevisiae*.

Apoptotic assays rely on evaluating the staining of cellular components in cells exposed to a particular treatment followed by flow cytometric tests. The most popular stains are Annexin V-FITC/PI and 7-AAD (Hernandez et al., 2016; Scariot et al., 2016). Annexin-V-FITC (AV), PI, and 7-AAD are membrane-impermeable molecules. When cells are dying from apoptosis, exposed phosphatidylserine on the outer plasma membrane early in this process. At later stages of apoptosis, cells only become permeable to PI; meanwhile, necrotic cells present an early breakage of the plasma membrane permeability barrier and almost no translocation of phosphatidylserine (Martin et al., 1995; Madeo et al., 1997) on the other hand, 7-AAD is a nucleic acid dye that penetrates only membrane-damaged cells.

Epoxiconzale has not been assayed in *Saccharomyces cerevisiae* using apoptosis tests. However, Šiviková et al. (2018) tested epoxiconazole in bovine lymphocytes. Authors reported that epoxiconazole could significantly affect cell cycle kinetics and induce apoptosis in cultured bovine peripheral lymphocytes. Furthermore, they confirmed an apoptosis mechanism thanks to the ladder-like pattern of DNA fragments in electrophoretic analysis when yeast is exposed to prolonged exposure at the highest concentrations (50 μg/mL and100 μg/mL) of epoxiconazole.

Amine fungicides treatment induces cell death by apoptosis in the yeast *Saccharomyces cerevisiae* (Hernández et al., 2016). Although the fungicide Fenpropimorph has not been evaluated in an apoptosis assay using yeast. Hernández et al. (2016) reported that Tridemorph, a similar amine-fungicide as Fenpropimorph, stimulated an apoptotic cell death by inhibiting vacuole acidification. They treated W303-1a cells with 3 µM tridemorph for five hours before Annexin V-FITC/PI flow cytometric tests.

Several studies have proven Mancozeb's pro-apoptotic effects on mammalian cells (Fitsanakis et al., 2002; Calviello et al., 2006; Domico et al., 2007). In yeast, there are two reports evaluating this condition. The first one comes from Dias and colleagues (2010), which used sub-inhibitory concentrations and low-oxygen fermentation growth conditions. The other from Scariot and co-workers (2016) used stains Annexin V-PE and 7-AAD followed by flow cytometry. The latter study combined these dyes to identify four stages of cells: normal, apoptotic, necrotic, and apoptotic/necrotic. Following MZ-treatment in the standard experimental conditions, they observed that 54 % of cells of the wild-type yeast strain were apoptotic (positive for Annexin V positive and negative for 7AAD). In contrast, these cells represented only 6.5 % of the *Δ*yca1 mutant strain. However, the treatment did not produce significant differences in the number of necrotic cells of wild-type and *Δ*yca1 mutant strains in the presence of MZ. Also, they reported a dose- and time-dependent reduction in cell viability of 95.2 % c.f.u. after six hours using 100 μM (26.6 mg/l MZ) MZ treatment.

7.1.2. Reactive Oxygen Species (ROS)

Oxygen is essential for most organisms on Earth; however, this element can also display toxic properties against the same individuals that use it during aerobic respiration. Thus, organisms have evolved a plethora of mechanisms to defend against reactive oxygen species (ROS), which are superoxide radical (O_2^-, hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^\cdot$) (Farrugia & Balsan, 2012). Exposure to ultraviolet (UV), heavy metals, irradiation, pesticides, xenobiotics, air pollutants, and other endogenous factors can generate significant ROS (Farrugia & Balsan, 2012).

Scariot et al. (2016) used wild-type and $\Delta yac1$ strains of *S. cerevisiae* to evaluate ROS accumulation in the exponentially growing phase before and after MZ treatment. They set three standard redox-sensitive probes to measure the accumulation: DH123, DHE, and H2-DCFDA. DH123 and DHE indicated a high concentration of intracellular ROS in control and mutant strains. On the other hand, MZ-treated cells stained with H2-DCFDA obtained a decreased fluorescence and thereby lowed ROS accumulation than the control reported by Dias et al. (2010). Interestingly, wild-type possessed a higher ROS production than mutants under MZ treatment because the median fluorescence intensity of DHE was bigger than DH123.

On the other hand, Dias and co-workers (2010) performed a chemogenomic study using subinhibitory treatment of Mancozeb; they concluded that MZ exerted similar activity as a thiol-reactive compound in yeast. Thus, their conclusions differ from Scariot and co-workers, who concluded that MZ acts as a ROS inducer. Conversely, Dias's conclusions might be

attributed to the experimental design used in his study. Dias's group used subinhibitory MZ concentrations and low-fermentation growth conditions instead of high MZ concentrations and aerobic growth conditions set by Scariot's group. It is noteworthy that the Scariot's concentrations are more similar to the ones used in agricultural applications. Therefore, high MZ concentrations also carry high manganese and zinc concentrations inside cells. Like other metals, these cause cellular dysfunctions, increasing ROS and inducing apoptosis (Domico, 2007; Bussche, 2011; Nargund, 2008; Pereira, 2008). Interestingly, both mechanisms of inducing ROS production are not incompatible since Mancozeb is a 'multi-site' fungicide. Hence, ROS production in yeast by an MZ treatment can be devoted to MZ acting as a ROS inducer or as a thiol-reactivity compound inside yeast cells (Scariot, 2016).

7.1.3. Mitochondrial bioassays

Studies performed in mesencephalic rat cells determined that mancozeb induced mitochondrial dysfunction (Domico et al., 2006). This previous report led Scariot and collaborators (2016) to measure the effect of MZ on the mitochondrial membrane potential of yeast using the mitochondria-specific voltage-dependent dye DiOC6. They found that MZ-treated wild-type cells (BY4741) and *Δ*yca1 (Y06233) isogenic mutant strain exhibited increased fluorescence compared to controls indicating that Mancozeb induces hyperpolarization of yeast mitochondrial membranes.

Surprisingly, Casalone et al. (2010) reported that the frequency of petite mitochondrial mutants in a yeast (wine yeast 1014, Industrial Yeasts Collection DBVPG) population exposed to a subinhibitory concentration of MZ is higher relative to that measured in a numerically similar not-exposed population. They attributed this increase to the intrinsic MZ resistance of petite cells and their capability to outgrow wild-type cells during overnight incubation in media with a subinhibitory concentration of MZ. Thus, they explained, the MZ-tolerance adopted by petite mutants could be explained by a retrograde response from dysfunctional mitochondria to the nucleus, resulting in the activation of Pdr3p, responsible for the expression of multidrug resistance transport proteins such as Pdr5p (Zhang and Moye-Rowley 2001) and Flr1p (Texeira et al. 2008; Dias et al. 2010).

7.1.4. Cell cycle evaluation bioassays

Hernández et al. (2016) showed that tridemorph could induce cell cycle arrest to a certain extent. Accumulating abnormal sterols has been associated with cell cycle perturbations (Marcireau et al., 1990; Fernández et al., 2002); thus, they also researched if cell cycle arrest co-occurred with apoptosis. Experiments using PI staining of permeabilized cells previously treated with tridemorph revealed that genomic DNA fragmentation occurs upon fungicide treatment with increased cells at the G1 phase of the cell cycle. Comparison between erg2 (JRY7773) and wild-type showed no differences in subG1 extent (below 4%) and only a slight accumulation of cells in G2/M phases of the cell cycle. Furthermore, the deletion of the only yeast metacaspase gene (YCA1) showed cells with no differential sensitivity toward tridemorph as assessed by PI-flow cytometry of permeabilized cells and the drop test assays. On the other hand, Scariot and colleagues (2016) evaluated MZ-treated and untreated wild-type and *Δ*yca1 yeast strains by flow cytometry followed by staining with the nucleic acid binding dye SYTO9. The authors reported no significant modification of the percentage of cells in the G1, S, and G2 stages, indicating that Mancozeb did not interfere with cell cycling.

It is undoubtedly that yeast-based assays provide enormous advantages regarding lower costs and less experimental time evaluating chemical toxicity (Papaefthimiou et al., 2004; Yasokawa & Iwahashi, 2010; dos Santos et al., 2012). Nonetheless, dos Santos and colleagues (2012) argued that bioassays relying solely on phenotypic endpoints often lack sensitivity and provide no mechanistic information. Therefore, deeper approaches like omics studies (chemogenomics, transcriptomics, and proteomics) allowed researchers to understand the yeast's integrally responses to xenobiotics (fungicides).

7.2. Omic studies with *S. cerevisiae*

7.2.1. Chemogenomic studies

Chemogenomics or Toxicogenomics refers to the study of biological functions of genes concerning toxic environmental stressors (Hamadeh et al., 2002; North & Vulpe, 2010). *S. cerevisiae* is one of the main tools for this field thanks to its long history of genetic manipulation studies that allowed researchers to systematically analyze and fully annotate the yeast genome, deciphering the function of each gene (Braconi et al., 2016). Furthermore, the use of yeast as an experimental model presents an advantage because many of yeast's genes have human orthologs. Hence, researchers can speculate about the conserved mechanisms in more complex eukaryotes.

Several studies have been conducted regarding pesticides' toxic effects on the *Saccharomyces cerevisiae* genome using a chemogenomic approach (Dias et al., 2010, Yadav et al., 2011; Gaytán et al., 2013a, b; Ušaj et al., 2014; Guan et al., 2020). Nonetheless, only two chemogenomic studies are testing the fungicide effects on Saccharomyces. These two studies tested mancozeb and epoxiconazole in 2010 and 2020, respectively (Dias et al., 2010; Guan et al., 2020).

Dias et al. (2010) identified 286 genes, including three essential genes, upon Mancozeb exposure to yeast strains and their deletions mutants conferring tolerance to MZ based on the high susceptibility of the corresponding deletion mutants toward this fungicide when compared to the parental strain. Furthermore, Gene ontology and genetic interaction analysis of datasets allowed them to cluster the genome response of yeast-treated with MZ into three main categories (oxidative stress response, carbohydrate/energy metabolism, and protein degradation) that confers Mancozeb tolerance in yeast (Table 4). Importantly, this study ratifies previous research highlighting a vast majority of up-regulation of downstream genes of the major oxidative stress regulator in yeast (Yap1p) and the roles of plasma membrane multidrug transporters of the major facilitator superfamily (FLR1 and TPO1) in MZ stress response. (Dias et al., 2010; Santos et al., 2009).

Functional genomic single-gene knockout mutant screening can be used to identify strains with increased or decreased sensitivity in the presence of chemicals, providing an unbiased assessment and deep insights into the potential toxicity mechanism of chemicals (Alfatah et

al., 2019; Guan and Zhang, 2017; Parsons et al., 2006; Xia et al., 2016). Furthermore, this type of screening enables the identification of specific genes to establish a direct interaction between chemicals and adverse effects (Guan et al., 2018; Reczek et al., 2017). Guan and colleagues (2020) evaluated the molecular toxicity mechanism of five conazoles (propiconazole, penconazole, tebuconazole, flusilazole, and epoxiconazole) using this knockout mutant screening approach in the model organism *S. cerevisiae*. They found that the five conazoles tended to have similar gene mutant fingerprints and toxicity mechanisms. Specifically, they identified a total of 173 (4.59%) responsive genes using three concentrations (IC50, IC20and IC10) of Epoxiconazole. These responsive gene set enrichment analyses showed that "Ribosome" and "cytoplasmic translation" were the common KEGG pathway and GO biological process terms. Additionally, conazoles also affected fatty acid synthesis because the "biosynthesis of unsaturated fatty acids" pathway was among the top-ranked KEGG pathways. Moreover, two genes, YGR037C (acyl-CoA-binding protein) and YCR034W (fatty acid elongase) were crucial fingerprints of conazoles because they played vital roles in conazole-induced toxicity. YGR037C (ACB1) and YCR034W (ELO2) have homologous genes in many organisms, including humans. Other studies have confirmed that YGR037C and YCR034W disturbing functions (i.e., disturbed lipid metabolism, fatty acid metabolism, and bile acid metabolism) are similar even in other organisms such as zebrafish, rats, and humans (Hermsen et al., 2012; Hester and Nesnow, 2008; van Dartel et al., 2011; Wang et al., 2017). Finally, Luckert et al. (2018) demonstrated that conazole treatment led to a statistically significant increase in cellular triglycerides in human HepaRG cells after 24 and 72 h using fluorescence-based AdipoRed assay (Luckert et al., 2018).

Table 4. *Saccharomyces cerevisiae* genes with their Human orthologs genes under a conazoles treatment (Guan et al. 2020)

Treated cell	Gene in *S. cerevisiae*	Human Homologous genes	References
Epoxiconazole	YGR037C (ACB1)	ACBD 4, ACBD5, ACBD7 and DBI	Guan et al., 2020
Epoxiconazole	YCR034W (ELO2)	ELOVL1, ELOVL2, ELOVL3, ELOVL4, ELOVL5, ELOVL6 and ELOVL7	Guan et al., 2020

Texeira et al. (2008) highlighted the role of a small network of transcription factors (YAP1 and RPN4) and plasma membrane multidrug transporters of the major facilitator superfamily (FLR1 and TPO1) that played an essential role in MZ stress response. A posterior chemogenomic study by Dias and coworkers (2010) identified 286 genes that protected MZ in *S. cerevisiae* [12]. They applied Gene ontology and genetic interaction analysis of this dataset which allowed us to highlight the role of oxidative stress response, carbohydrate/energy metabolism, and protein degradation in Mancozeb tolerance. Likewise, Dias et al. (2010) and Santos et al. (2009) studies showed that the vast majority of the up-regulated genes under MZ stress were downstream targets of the primary oxidative stress regulator in yeast (Yap1p).

Casalone et al. (2010) reported that the frequency of petite mitochondrial mutants in a yeast (wine yeast 1014, Industrial Yeasts Collection DBVPG) population exposed to a subinhibitory concentration of MZ is higher relative to that measured in a numerically similar not-exposed population. They attributed this increase to the intrinsic MZ resistance of petite cells and their capability to outgrow wild-type cells during overnight incubation in media with a subinhibitory concentration of MZ. Thus, they explained, the MZ-tolerance adopted by petite mutants could be explained by a retrograde response from dysfunctional mitochondria to the nucleus, resulting in the activation of Pdr3p, responsible for the expression of multidrug resistance transport proteins such as Pdr5p (Zhang and Moye-Rowley 2001) and Flr1p (Texeira et al. 2008; Dias et al. 2010).

Table 5. *Saccharomyces cerevisiae* cluster genes with their Human orthologs under a mancozeb treatment.

Fungicide applied	**Gene Cluster**	**Genes**	**Human orthologues**
Mancozeb	Carbohydrate Metabolism	CCR4, FPS1, FYV10, GVR2, GPB2, GPH1, LPD1, NGG1, PFK1, PYC1, REG1, RPE1, SFA1, SNF1, TUP1, VID22, YVH1, ZWF1	CNOT6, CNOT6L, AQP9, AQP7, AQP3, APQ10, APQ7P2, MAEA, PYGB, PYGL, PYGM, DLD, PFKL, PFKM, PFKP, PC, RPE, ADH1A, ADH4, ADH5, ADH1B, ADH7, PRKAA1, DUSP12, H6PD, G6PD
Mancozeb	Mitochondrial Function	ATP1, ATP12, ATP15, ATP5, CBP6, FZO1, MGM1, MHR1, MRH1, MRPL35, MRPL36, MRPS28, MRPS35, MSF1, MSY1, NDE1, PET8, RRF1, RSM27, RTG1,	ATP5A1, ATPAF2, ATP5E, ATP5O, FARS2, YARS2, SLC25A26, MRRF,

		RTG2, SAM37, SHE9, UGO1	
Mancozeb	Multiple Drug Resistance (MDR)	FLR1, RPN4, TPO1, YAP1	—
Mancozeb	Oxidative Stress Response	CCS1, COQ1, GLR1, GSH1, GSH2,, RIB4, SOD1, SOD2, YAP1	PDSS1, GSR, GCLC, GSS, SOD1, SOD2
Mancozeb	Protein Degradation	<u>Ubiquitin System</u> BR1, BRE5, DOA1, DOA4, DOC1, RAD6, RTT101, UBI4, UBX4	RNF40, RNF20, PLAA, ANAPC10, UBE2A, UBE2B, RPS27A, ASPSCR1
		<u>Proteasome system</u> NAS2, RPN4, SEM1, UBP6	PSMD9, SHFM1, USP14
		<u>UPR, ERAD, MVB, and VPS systems</u> ATG11, BRO1, BST1, CLC1, EPS1, GTR2, HSE1, MON1, MON2, PBI2, PEP12, PEP3, PEP5, SNF7, SWF1, VPS1, VPS20, VPS25, VPS27, VPS3, VPS36, VPS4, VPS41, VPS74, VPS8	PTPN23, PGAP1, CLTA, CLTB, RRAGC, RRAGD, STAM, STAM2, MON1A, MON1B, MON2, STX7, STX12, VPS18, VPS11, CHMP4A, CHMP4B, CHMP4C, ZDHHC4, DNM1L, CHMP6, VPS25, HGS, VPS36, VPS4A, VPS4B, VPS41, GOLPH3, GOLPH3L, VPS8
		Other MKX7, UBA3, YME1	UBE1C, YME1L1

7.2.2. Transcriptomic studies

Organisms can cope with changes in the environmental conditions by suddenly switching mRNA expression, which can be quantified by DNA microarray technology, establishing potential functional relationships (Braconi et al., 2016). *Saccharomyces cerevisiae* has been evaluated under several pesticides (lindane, atrazine, 2,4-dichlorophenoxyacetic acid (2,4-D), dithiocarbamates and anilino pyrimidines) to quantify transcriptomic responses helping hypothesize about the mechanisms that underlie these treatments. Yeast has also been used as a model to uncover the toxicity associated with a widely used fungicide, especially

dithiocarbamates (mancozeb, thiuram, zineb, and maneb) and pyrimethanil. According to Teixeira and colleagues (2008), FLR1, transcription factor (encoding for a plasma membrane antiporter involved in multidrug resistance), exerts a strong activation during mancozeb-induced yeast growth adaptation that was utterly dependent on Yap1p (oxidative stress) and partially dependent also on Rnr4p (proteasome gene expression), Yrr1p or Pdr3p (pleiotropic drug resistance). On the other hand, Gil and coworkers (2014) carried out a transcriptomic study focused on two sublethal concentrations of PYR that inhibited yeast growth by 20%(IC20, 20 mg/L) or 50% (IC50, 50 mg/L) compared with control cells not exposed to the fungicide. They found increased expression of the whole set of genes assigned to arginine biosynthesis (from ARG1 to ARG11 and CPA1/CPA2) in the microarray data.

Furthermore, other gene categories related to sulfur amino acids metabolism, energy conservation, antioxidant response, and multidrug transport presented responses upon exposure to PYR. Authors selected by qRT-PCR ARG3, ARG4, ARG5, ARG6, ARG8, and MET28 genes as indicators of toxicity in the yeast upon PYR exposure. The selection was based on the consistent transcription variation profiles of the genes, which correlated well with the fungicide's concentration-dependent inhibition of yeast growth. PYR concentration inhibited yeast growth by only 20% produced modifications of the earlier genes between 4.0-and6.1-fold compared with control cells. Thus, the authors suggested these five genes may potentially incorporate in yeast-based cellular reporters or biosensors for biomonitoring or preliminary screening of fungicide toxicity due to offer a more sensitive approach than the ones based only on phenotypic endpoint assays (Gil et al., 2014). Finally, the authors reported that many pyrimethanil-responsive genes encoded proteins sharing significant homology with proteins from phytopathogenic fungi and ecologically relevant higher eukaryotes (Gil et al., 2014).

7.2.3. Proteomic Studies

Proteomics main objective is to analyze fluctuations in protein production or their post-translational modification (PTM), helping to clarify how changes at the genome level are translated into changes in fundamental molecular effectors (Braconi et al., 2016). Furthermore, proteomics analyses deliver biomarkers and essential proteins involved in molecular responses to xenobiotics, which can be used for environmental biomonitoring. Thus, biomarkers' modulation relates to practical impact on the physiology of organisms (Trapp et al., 2014);

nonetheless, reports of a systematic application of environmental biomarkers on a large scale are very scarce. Their use is limited to predictions at a laboratory scale.

Proteomics is suitable for ecotoxicological assessments. Several studies have been conducted to test pesticide treatments on *Saccharomyces cerevisiae* (Texeira et al., 2006; Braconi et al., 2009; Santos et al., 2009; Braconi et al., 2010). Tribenuronmethyl, glyphosate, fenoxaprop-P-ethyl2,4-Dichlorophenoxyacetic acid (2,4-D), and Mancozeb are the reported chemical pesticides tested in S. cerevisiae to obtain a global mechanical response of the yeast to xenobiotics.

Santos et al. (2009) analyzed the global response to mancozeb in yeast by quantitative proteomics using 2-DE means. Authors selected_target genes (e.g. TSA1, TSA2, SOD1, SOD2, AHP1, GRE2, GRX1, CYS3, PRE3, PRE6, PRE8, PRE9, EFT1, RPS5, TIF11, HSP31, HSP26, HSP104, HSP60, HSP70-family) and main transcription activators (e.g. Yap1, Msn2/Msn4, Met4, Hsf1, Aft1, Pdr1, Skn7, Rpn4p, Gcn4). They found that mancozeb-induced expression changes related to *Saccharomyces'* response to stress, specifically oxidative stress, protein translation initiation and protein folding, disassembling of protein aggregates and degradation of damaged proteins.

Saccharomyces cerevisiae possesses many signaling pathways, and their molecular components are substantially conserved among eukaryotic organisms. Additionally, the *S. cerevisiae*'s protein-protein interaction network is the best understood and most complete of all eukaryotes, allowing insights into human diseases (Hsu et al., 2007). Regarding fungicides, researchers have related global yeast response to mancozeb toxicity to potential indications of toxicity in humans. Therefore, protein interaction maps have been constructed from the analysis of global yeast response that confirmed percentages of 53% to 70% of the yeast-altered-expression-proteins have human orthologs (Santos et al., 2009; Dias et al., 2010)

Figure 16 displays protein-protein interaction networks where yeast genes are compared with their human orthologs (Dias et al., 2010). For example, the yeast VMA4 and VMA13, identified as determinants of mancozeb tolerance, have their corresponding human orthologs ATP6V1E1 and ATP6V1H, which compose the human V-ATPase (Figure 16). Sennoune et al. (2004a, 2004b) demonstrated that human V-ATPases are overexpressed in many types of metastatic cancers, and this altered expression positively correlated to their potential for invasion and metastasis. Three other human genes involved in tumor development and invasiveness, PAK1, MAP2K1, and LCP1 (L- plastin), are orthologues of the yeast genes CLA4, PBS2, and SAC6 (Figure 16), also identified as determinants of mancozeb tolerance (Klemke et al., 2007; Wang et al., 2006). One of the Pak1-interacting proteins is Cdk5, the

human orthologue of Pho85p, whose deregulation has also been implicated in neurodegenerative pathologies, including Parkinson's disease (Dhariwala and Rajadhyaksha, 2008). Notably, oxidative stress-related genes are also conserved between yeast and humans. Yap1p, the most significant gene determinant of yeast tolerance to mancozeb, and its human orthologues Junp and Jdp2p (Moye-Rowley et al., 1989) exhibit similar interaction networks. The latter are activated during acute and chronic phases of many neurodegenerative diseases (Shaulian and Karin, 2002). Junp and Jdp2p can recognize proteins belonging to the RNA polymerase and the chromatin-histone remodeling complexes (Figure 16). GCLC, GSS, and GSR, the human orthologues of GSH1, GSH2, and GLR1, are also involved in the recovery of reduced glutathione (GSH), whose levels are a determinant factor in the prevention of Parkinson's disease onset (Riederer et al., 1989; Sofic et al., 1992). ||| Superoxide dismutase 1 and 2 (SOD1 and SOD2) are the human orthologues of yeast Sod1p and Sod2p, respectively. According to Ihara et al. (2005), SOD1 may play a role in the onset and progression of Parkinson's disease, and its induction may act neuroprotective. The corresponding human ortholog of yeast Kar2p is HSPA5 which plays a critical role in the regulation of protein synthesis, folding and trafficking in the ER stress response. Joo et al. (2007) stated the importance of ER stress response activation by studying human lung carcinoma cells. They stated that to lower the protein synthesis, transport for degradation in the ubiquitin/proteasome system of misfolded or unfolded proteins, induction of chaperone synthesis to increase folding capacity and induction of apoptosis.

Finally, three of the identified determinants of mancozeb stress tolerance in yeast coding for subunits of the proteasome complex (NAS2, SEM1, and UBP6) have a corresponding human orthologue (PSMD9, SHFM1, and USP14, respectively). Despite the exact underlying mechanism being unclear, Dahlaman (2007) hypothesized that an age-related decrease in proteasome activity weakens cellular capacity to remove oxidized modified proteins and favors the development of neurodegenerative diseases (Dahlmann, 2007).

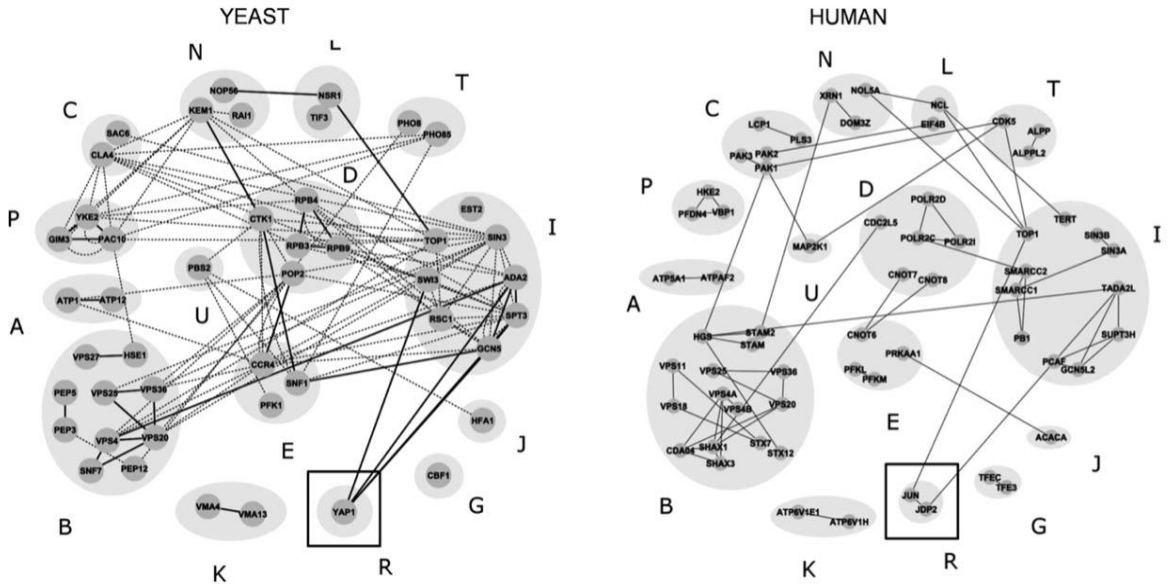

Figure 16. Physical interactions of the yeast genes were identified as determinants of mancozeb stress tolerance and the protein interaction map of the corresponding human orthologs. Yap1p and its human orthologs are highlighted in black squares. The letters correspond to the gene clusters generated by Dias et al. 2010 A: Mitochondrial function; B: UPR, ERAD, MVB and VPS systems; C: Cytoskeleton, cell cycle, budding, mating and sporulation; D: RNA polymerase and mediator complexes; E: Carbohydrate metabolism and regulation; F: Unknown functions; G: Nitrogen, nucleotide, amino acid metabolism and regulation; H: Ubiquitin, proteasome and protein degradation; I: Histone and Chromatin modification; J: Lipid, fatty acid and ergosterol metabolism; K: pH homeostasis; L: Ribosome and translation; M: Cell wall biosynthesis and biogenesis; N: RNA processing and modification; O: Oxidative stress response; P: Protein folding and modification; Q: Nuclear trafficking; R: Multidrug resistance; S: Metal ion homeostasis; T: Phosphate homeostasis; U: Osmotic stress response.

8. Discussion

Black Sigatoka is the most destructive disease affecting up to 50% of yield in commercial banana plantations. Its deleterious effects threaten the production of *Musa* spp in all the banana-growing regions of the world. In Ecuador, the production of bananas constitutes a significant pillar of the national economy. Additionally, *Musa* spp represents an essential commodity for local and international markets. Researchers have proposed many methods to control BLSD in commercial plantations as well as non-commercial plantations of bananas. The conventional approaches to face BLSD have been the total or partial defoliation of affected leaves and traditional breeding. Defoliation helps stop the spread of ascospores or conidia outside infected plantations. However, it needs the application of other cultural strategies

(composting, drainages, and soil nutrition and pest control) to guarantee the effective non-dispersal of the inoculum.

On the other hand, traditional breeding has come out with several IFHA banana hybrids, which can be resistant to abiotic and biotic (BLSD); however, commercial features have been compromised. For that reason, Genetic Engineering techniques (transformation of resistance genes, overexpression of pathogenesis-related protein (PR) and RNAi-mediated gene silencing) have been applied to *Musa* spp to create resistant species. Many plants extract from *Momordica charantia*, *Senna reticulata*, and *Thevetia peruviana* (Pers.) represent novel and promising solutions for controlling BLSD (Paola, 2006; Dooh et al., 202; Seydou et al., 2021). Nonetheless, this approach constitutes only a preventive treatment tested in vitro. Hence, more research is devoted to finding and producing more new compounds with their corresponding field testing to ensure validated solutions against BLSD.

Chemical fungicides have accompanied human civilization for thousands of years. These compounds have mainly been devoted to protecting important crops that undergo destructive infection by phytopathogenic fungi. Both protectant and systemic fungicides have two alternative modes of action. Protectant fungicides only covered the superficial plant tissues preventing the fungus from establishing on its host. Meanwhile, systemic fungicides penetrate plant tissues exerting a more specific mode of action, targeting more efficiently the action of the pathogenic fungus. Both protectant and systemic have inside them other important fungicides groups such as Amines, Anilino-pyrimidines, Dithiocarbamates and Triazoles that also protect essential products derived from agricultural activities. These chemical fungicides represent the most popular and efficient way to control BLSD worldwide, including in Ecuador (Sabando, 2015; Franco, 2016; Portilla, 2017). Mainly, these fungicides are applied to banana plantations into cocktails by light aircraft before the beginning of the rainy season (December-May) in Ecuador. FRAC recommendations have led governments to foster the use of cocktail formulations that combine two or more fungicides (protectant or systemic), especially mancozeb, benomyl, chlorothalonil and tridemorph (FRAC, 2018).

Nevertheless, the continued and uncontrolled aerial sprays have brought some drawbacks for the environment and the workers or villagers near Musa spp plantations. Pathogen resistance, pesticide residues, environmental pollution and severe health conditions constitute the severe drawbacks found mainly in the three most-productive-banana-provinces of Ecuador: El Oro, Guayas and Los Ríos. Additionally, North American and European markets demand more sustainable or organic products; Hence, searching for more eco-friendly solutions, such bio fungicides, to combat BLSD is necessary to maintain the local and

international economy as well as food safety, ensuring wellness for both the environment and people (Chang, 2021).

Saccharomyces cerevisiae is a versatile organism that has been widely used for performing toxicity tests, detection of xenobiotic substances and bioremediation procedures (Knight et al., 2004; Schofield et al., 2007; Esteve et al., 2009; Abigail et al., 2013; Nguyen et al., 2017; Hernández Castellanos, 2018; Han et al., 2019; Gong et al., 2020; Liang & Han, 2020; Mollocana, 2020). Regarding fungicides, *S. cerevisiae* has been submitted to pesticide treatment to evaluate its physiologic, genomic, proteomic and transcriptomic response (Dias et al., 2010; Guan et al., 2020; Gil et al., 2018).

Dias and colleagues (2010) and Scariot and co-workers (2016) found that Epoxiconzaole, Mancozeb and Azoles (Fenpropimorph) induce apoptosis in wild-type of yeast. Surprisingly, mutant yeast strains such *Δyac*1 exposed to MZ treatment presented a lower percentage of apoptotic cells, indicating the critical role of the metacaspase Yca1 in the programmed death in yeast and revealing the potential induction of MZ to apoptosis in yeast.

Reactive Oxygen species (ROS) generates in organisms when they are exposed to a deleterious agent (UV radiation, heavy metals, pesticides, air pollutants) (Farrugia & Balsan, 2012). Studies assaying ROS production with Mancozeb where MZ can exert ROS inductor and a thiol-reactivity compound (Scariot et al., 2016). Meanwhile, Epoxiconazole, Fenpropimorh, and Pyrimethanil have not been assayed for this type of bioassay. This dual feature of MZ can originate from its multisite activity mainly as a protectant fungicide. Additionally, to its ROS activity, MZ produces hyperpolarization of yeast mitochondrial membranes, which researchers hypothesized are relatable to an apoptotic mechanism.

Scientists have established the direct relationship between mitochondrial dysfunction and apoptosis when mammalian or yeast cells are exposed to oxidative stress created by a xenobiotic. Therefore, Casalone and Scariot et al. (2016) studies exposed and confirmed mitochondrial dysfunction in yeast when the cells are under fungicide MZ-treatment. Nonetheless, the yeast mutants, *Δ*yac1 for Scariot et al. (2016) and petite yeast cells for Casalone can resist mancozeb treatment. The *Δ*yac1posses resistance to MZ due to its lack of the metacaspase Yac1 important for the apoptotic mechanism; meanwhile, the latter mutants express Pdr3p, which ultimately activates Pdr5p and FLR1which are multidrug resistance transport proteins.

9. Concluding Remarks

Black Sigatoka Streak Disease severely affects commercial banana plantations worldwide. BLSD represents a severe problem for Ecuador, in which the production of bananas constitutes a significant pillar of the national economy. The chemical management with protectant and systemic fungicides has helped to maintain a stable production of Musaceae in the country. The fungicide management uses commercial formulations of Epoxiconazole, Fenpropimorph, Mancozeb, and Pyrimethanil that sometimes are mixed into cocktail formulations and distributed over large commercial plantations by aerial spraying. Nonetheless, the continuous and uncontrolled sprays have raised concerns about generating pathogen-resistant strains of *Mycosphaerella fijiensis*, eco-toxicological contamination and health conditions on people that work and live near these plantations. For that reason, the implementation of public policies that foster the investigation of both new alternatives to the current chemical fungicides and affordable and easy-to-use technologies for the control of commercial banana fungicides in Ecuador is evident.

The physiological and omics responses that Epoxiconazole, Fenpropimorph, Mancozeb, and Pyrimethanil produce on *Saccharomyces cerevisiae* constitute hallmarks for future research in higher eukaryotes. Furthermore, these yeast-cellular reporters must be considered to be incorporated on future biosensors for biomonitoring or preliminary screening of fungicide toxicity, in line with the 3Rs attempt to “reduce, refine, and replace” animal models in toxicity testing.

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