The origin, versatility and distribution of azole fungicide resistance in the banana black Sigatoka pathogen *Pseudocercospora fijiensis*

Pablo A. Chong Aguirre

Thesis committee

Promotors
Prof. Dr G.H.J Kema
Professor of Tropical Phytopathology
Wageningen University

Prof. Dr P.W. Crous
Professor of Evolutionary Phytopathology
CBS-KNAW Fungal Biodiversity Center, Utrecht

Other members
Dr J.J. Stoorvogel, Wageningen University

Prof. Dr B.J. Zwaan, Wageningen University

DrB.A. Fraaije, Rothamsted Research, Harpenden, United Kingdom

Prof. Dr C.M.J. Pieterse, Utrecht University

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Pablo A. Chong Aguirre
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Chapter 1

General introduction and outline of the thesis

Black Sigatoka disease of banana, caused by *Pseudocercospora fijiensis*

Banana, the top fruit of the world and an important staple

The genus *Musa* contains banana plants with edible fruits, which are among the oldest domesticated crops. Archaeological studies indicated that the domestication process of bananas and plantains probably started around 7,000 years ago in Southeast Asia (D'Hont *et al.* 2012; Perrier *et al.* 2011). The modern geographical distribution of *Musa* spp. includes the tropical and subtropical areas of the Americas, Africa, the Caribbean Islands, Melanesia, the Pacific islands and Southeast Asia (mainland and islands). Bananas are among the most important crops worldwide and rank highly on the list of valuable agricultural commodities (Ploetz 2000; Ploetz *et al.* 2015). The 2012 – 2013 banana market review from the Food and Agriculture Organization of the United Nations (FAO), stated that the global production of bananas was around 106.7 millions of tons.

The global banana export reached 16.5 million tons with a gross production value of US $29.7 billion (FAO 2014a, b). Ecuador is the largest exporter in the world, exporting 5.19 million tons (MT) in 2012, followed by the Philippines with 2.6 MT, Guatemala with 1.98 MT, Costa Rica with 1.88 MT and Colombia with 1.83 MT (FAO 2014a, b) (Figure 1). The main importers of banana in 2012 were the United States of America and countries of the European Union with 4.4 MT and 4.3 MT, respectively (FAO 2014a). Other important markets are Russia, Japan and China (FAO 2014b).

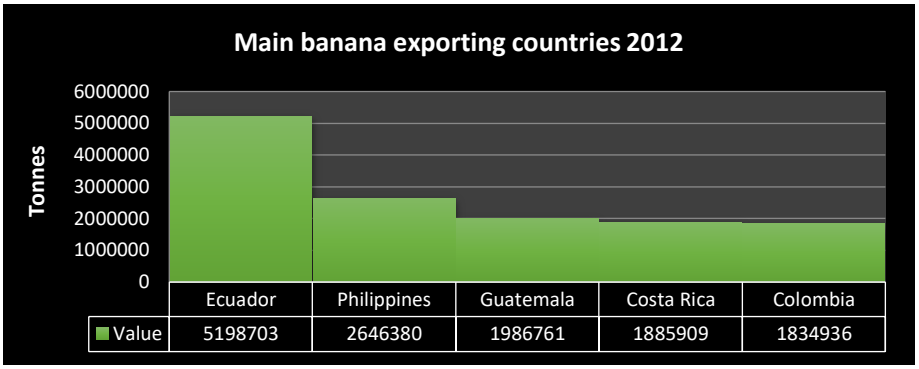

Figure 1. Main banana exporting countries in 2012, data taken from FAOSTAT.

The global banana export represents only 15.5% of the total banana production. The remaining 84.5% represents the banana production for domestic markets and local consumption. This underscores the importance of the banana fruit as a major fruit in many tropical and subtropical countries. Cooking bananas are a starchy staple food crop for approximately 500 million people (Collins 2014), such as in Uganda where it is a major staple food as well as an important cash commodity for communities (Shively & Hao 2012). Bananas are also very important in the local markets of Asia. Virtually all banana production from India and China are destined for local markets (Ploetz *et al.* 2015). Many banana varieties for local consumption are nutritious - rich in minerals and vitamins A, C and B6 - relative cheap and easy to produce (Ekesa *et al.* 2012; INIBAP 1998). In developing countries in Latin America, the banana trade is an important source of income. For example, in Ecuador, 95% of the total production is exported and represents almost 60% of the agricultural gross domestic product (GDP; US$ 1.9 billion in 2009). In fact, bananas are the second export product of Ecuador after oil (Vega 2011). They are an important factor in the economy, strengthening the rural communities in the coastal region (Vega 2011). In Ecuador, the majority of producers are smallholders with 71% working on up to 20 hectares. In 2009, 2.5 million Ecuadorians,

representing 17.5% of the population, were directly or indirectly involve in the banana industry (Vega 2011). Different banana and plantain varieties are easy to spot in local markets, where they are cheap and provide an accessible source of energy, minerals and vitamins. A similar pattern emerges in African developing countries. From the total agricultural output of Uganda, 25% of the value concerns bananas and top-ranks the *per capita* consumption in the world (0.70 $kg.person^{-1}.day^{-1}$) (Shively et al 2012). Comparable developments are observed in the eastern parts of the Democratic Republic of Congo where bananas (plantains and cooking bananas) constitute the second main starchy staple food after cassava, with a consumption rate of around 0.37 - 0.48 $kg.person^{-1}.day^{-1}$ (Ekesa *et al.* 2012).

Banana plants are monocotyledons of the order Zingiberales. Most edible bananas belong to the *Eumusa (Musa)* section of the family *Musaceae* with seedless diploid, triploid or tetraploid genetic configurations derived from two founding species, *Musa acuminata* (the wild diploid, fertile A-genome donor) and *M. balbisiana* (the wild diploid, fertile B-genome donor), either alone or in various combinations (D'Hont *et al.* 2012; Perrier *et al.* 2011). The subgroup Cavendish comprises triploid sterile hybrids (AAA) derived from *M. acuminata* and is the most significant banana group representing 28% of global banana consumption and nearly all of the worldwide banana export. The plantain subgroup (AAB), important in African and Latin America, is accountable for 21% of the global fruit production (Ploetz *et al.* 2015). Commercial bananas are typically produced in large monocultures, which results in increased disease threats. Smallholders frequently grow different banana cultivars in mixed cropping systems resulting in complicated management, but with significantly lower disease pressure (Ploetz *et al.* 2015; Zadoks & Schein 1979). Like in any agricultural production system, but particularly in perennial crops, many abiotic and biotic factors as well as managerial activities affect banana production, including soil structure (physical and chemical), irrigation, fertilization, drainage systems, pesticides, fruit bag covering, bunch support, debudding and dehanding. Many

smallholders lack the resources to control these factors (Ploetz *et al.* 2015). Despite regional fluctuations, the most important diseases in banana crops are caused by fungi followed by - in descending importance order – bacterial and viral disease, nematode and insect pests (Ploetz *et al.* 2015).

Globally, the most important disease in banana production is the so-called black Sigatoka, or black leaf streak disease, caused by *Pseudocercospora fijiensis* (Morelet) (Deighton 1976), previously *Mycosphaerella fijiensis* Morelet (1969) (Churchill 2011b). Other important diseases are Panama disease caused by *Fusarium oxysporum* f. sp. *cubense* (FOC), yellow Sigatoka disease caused by *Pseudocercospora musae*, anthracnose caused by *Colletotrichum musae*, Banana Streak Virus (BSV) and the burrowing nematode *Radopholus similis* (Blomme *et al.* 2011; Ploetz *et al.* 2015). The relatively rapid, long-distance dissemination of diseases is thought to be associated with anthropogenic movement of infested material, specially by "suckers" (Ploetz *et al.* 2015). Banana suckers are lateral shoots developing from the rhizome of the mother plant that are used to vegetative propagate the plant by the growers. Before the era of tissue culture, this was the one and only way to reproduce the plant and it greatly contributed to the global dissemination of FOC (Ordoñez *et al.* 2015). The currently most important re-emerging banana disease is FOC caused by the genetic lineage vegetative compatibility group 01213, colloquially called Tropical Race 4 (TR4). Other emerging diseases are the bacterial diseases, namely Banana Xanthomonas Wilt (BXW) and blood disease caused by *Ralstonia haywardii* subspecies *celebensis*. These emerging diseases and some other important diseases like eumusae leaf spot (*Mycosphaerella eumusae*), freckle (*Phyllosticta maculata and allied species*) (Wong *et al.* 2012), banana lesion nematode (*Pratylenchus goodeyi*) and Banana Bunchy Top Virus (BBTV) have relative narrow geographical distributions, but may incur major losses (Ploetz *et al.* 2015). For the majority of these diseases, effective quarantine measures and the use of clean seed material are the only

available and hence, essential measures to reduce their dispersal. Other diseases - primarily the foliar blights, including the Sigatoka complex - and (insect and nematode) pests are manageable by using pesticides. However, reducing efficacies are a major concern as the major commercial banana varieties - including Cavendish - are (very) susceptible to a plethora of diseases (Ploetz *et al.* 2015).

The importance of black Sigatoka disease

Among the banana diseases, black Sigatoka is by far the major problem in the banana industry, causing serious leaf defoliation and indirect post-harvest fruit quality problems due to premature ripening of the fruit, turning it unacceptable for export (Ploetz 2000). The main control measure involves frequent fungicide applications, which has a very high environmental and economic burden (Risède *et al.* 2010). As such, black Sigatoka has a major effect on subsistence production of banana and plantain since most of the smallholders are unable to afford these fungicides (Ploetz 2000). Meanwhile the public opinion, debating the fungicide usage and the increasing negative environmental impacts, demands safer food and environmental friendly crop management. This justified demand has an increasing impact on global exporting regulations, directing towards reduced pesticide use in commercial banana production (Risède *et al.* 2010), which primarily comprises the highly susceptible Cavendish type monocultures.

Hence, one of the major issues in black Sigatoka control has been the excessive and unplanned fungicide use in many banana farms worldwide. This uncurbed use facilitated resistance development in the pathogen population, thereby reducing the efficacy of disease management and hence, maximizing the number of fungicide applications, such as presently in Costa Rica. In the 1990's, 30 applications per year were sufficient for disease control, but in 2007 the frequency had increased to over 50 treatments. Another example is Cameroon with 12

treatments increasing to nearly 50 applications per year between 1990 and 2008 (Lapeyre *et al.* 2010b). In Ecuador, the average number of applications was 10 – 12 in the 1990's, which increased up to approximately 30 applications per year in 2016 (CIBE, unpublished data). Consequently, these excessive fungicide applications do contribute to negative impacts on the environment and occupational health of workers in banana plantations and rural villages nearby, as mentioned in the World Health Organization report (Beaglehole *et al.* 2003; van Wendel de Joode *et al.* 2016). In 2006-2008, the international project 'Pesticide Reduction Program for Bananas (PRPB)' sponsored by the Common Fund for Commodities and coordinated by Wageningen University and Research, analysed global pesticide use in banana producing countries (Risède *et al.* 2010), which revealed that the majority of the currently applied fungicides is targeting *P. fijiensis*. In addition, a clear correlation was detected between black Sigatoka incidence, annual rainfall (more rain = more *P. fijiensis* spores = more fungicide applications) and the risk of reduced efficacy of fungicides, which forced farmers to shift to contact fungicides. Since contact fungicides lack a curative effect their application frequency is higher, which increases the chemical load in banana production (Risède *et al.* 2010). This vicious circle of required intensification of fungicide applications and increasing resistance in *P. fijiensis* populations towards systemic fungicides threatens fruit production and underscores the need for new molecules and application strategies to sustainably manage black Sigatoka in banana.

Disease symptoms

P. fijiensis symptoms start to appear 14 to 20 days after inoculation with red-brown specks (~0.25 mm diameter) at the lower leaf surface (Long 1979; Marín *et al.* 2003). These specks rapidly enlarge into reddish-brown streaks running parallel to the leaf veins that then develop into larger dark-brown to black composite streaks, which are indeed visible at both leaf

surfaces, but appear larger on the abaxial side (Long 1979). The streaks eventually form fusiform or elliptical spots that coalesce and form a water-soaked border with a yellow halo that eventually merge to cause extensive leaf necrosis (Figure 2). The time period from the first symptom to the streaks and subsequently necrotic spots varies depending on the cultivar and the severity of the infection (Marín *et al.* 2003). The symptom description of *P. fijiensis* infection is summarized in Tables 1 and 2 (Fouré 1985; Marín *et al.* 2003; Meredith & Lawrence 1969).

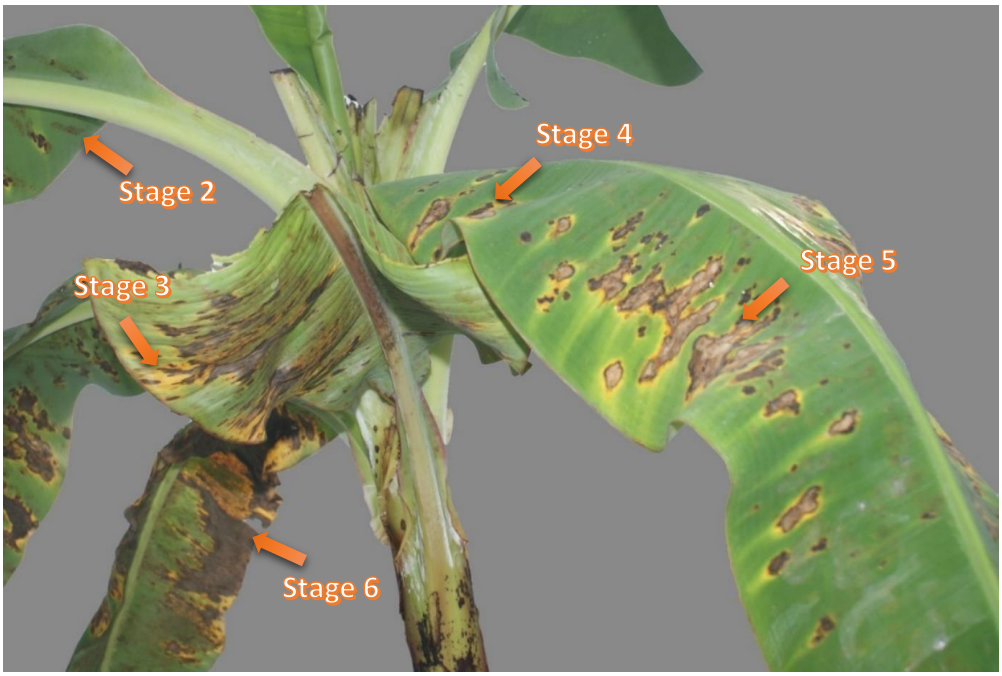

Figure 2. Banana plant infected with *Pseudocercospora fijiensis* in a greenhouse experiment. The plant shows the typical symptoms of the disease, streaks, elliptical necrotic lesions with water-soaked border and a chlorotic yellow halo and extensive necrosis. Mark in arrows are some of the stage of the disease based on Fouré description (Fouré 1985).

Table 1. Black Sigatoka symptoms on banana (Meredith & Lawrence 1969).

Common name	Stage	Description
Speck	Stage 1	First visible lesion. Reddish brown specks in the lower surface of the leaf (<0.25 mm). Abundant near the margin of the leaf. Usually first appear in the third, fourth or older leaves.
First Streak stage	Stage 2	Streaks elongate to form narrow reddish brown streaks up to 20 mm long and 2 mm wide. The long axis is parallel to the leaf venation. Frequently they are densely aggregated in a band several centimetres wide. Specks that overlap, form large, compound streaks.
Second Streak stage	Stage 3	The streaks may elongate slightly. The colour changes from reddish brown to dark brown or almost black. They are clearly visible in the upper surface of the leaf.
First Spot stage	Stage 4	The streaks broaden and become fusiform or elliptical. The spot is characterized by the development of a light brown, water-soaked border around the spot.
Second Spot stage	Stage 5	The dark brown or black central area of the spot becomes depressed and the water-soaked border becomes more pronounced. A slight yellowing of the leaf tissue surrounding the water-soaked border may occur.
Third Spot stage	Stage 6	The centre of the spot dehydrates, becoming light grey or buff-coloured and further depresses. The spot is bordered by a well define dark brown or black rim. There is a bright yellow transitional zone between the spot and the green healthy leaf tissue. After the leaf collapsed and withered, spots remain clearly visible.

Table 2. Black Sigatoka symptoms describe by Fouré taken from Marín et al 2003 (Fouré 1985; Marín *et al.* 2003)**.**

Common name	Stage (Fouré)	Description
Mark	Stage 1a	Depigmentation mark (whitish or yellow). Only on lower surface
Speck	Stage 1b	Red-brown speak on lower surface of the leaf
Dash/Lesion	Stage 2	Red-brown streaks on both side of the leaf surface
Streak	Stage 3	Wider streaks. Colour starts changing from red to dark brown.
Spot	Stage 4	Dark brown (lower) to black (upper) spots.
Burn	Stage 5	Black spot with chlorotic halo. Lesion is slightly depressed.
Necrosis	Stage 6	Centre of the spot dries out and becomes whitish to grey. Spot is surrounded by a dark brown to black border and further depressed.

The Pseudocercospora fijiensis life cycle

The life cycle of *P. fijiensis* starts with leaf infection by either ascospores or conidia. After a period of epiphytic growth of two to three days, germ tubes penetrate the leaf through the stomata (Lapeyre *et al.* 2010b). The first symptoms will generally appear 14-20 days after penetration of the leaf. Conidia form at early stages (stage 2 to 4) of the disease development and are splash-dispersed over short-range distances. Ascospores develop at stage 6 and become airborne and show long-range distance dispersal up to an estimated mean distance of 614 meters with a maximum distance of 1000 meters (Lapeyre *et al.* 2010b; Rieux *et al.* 2014).

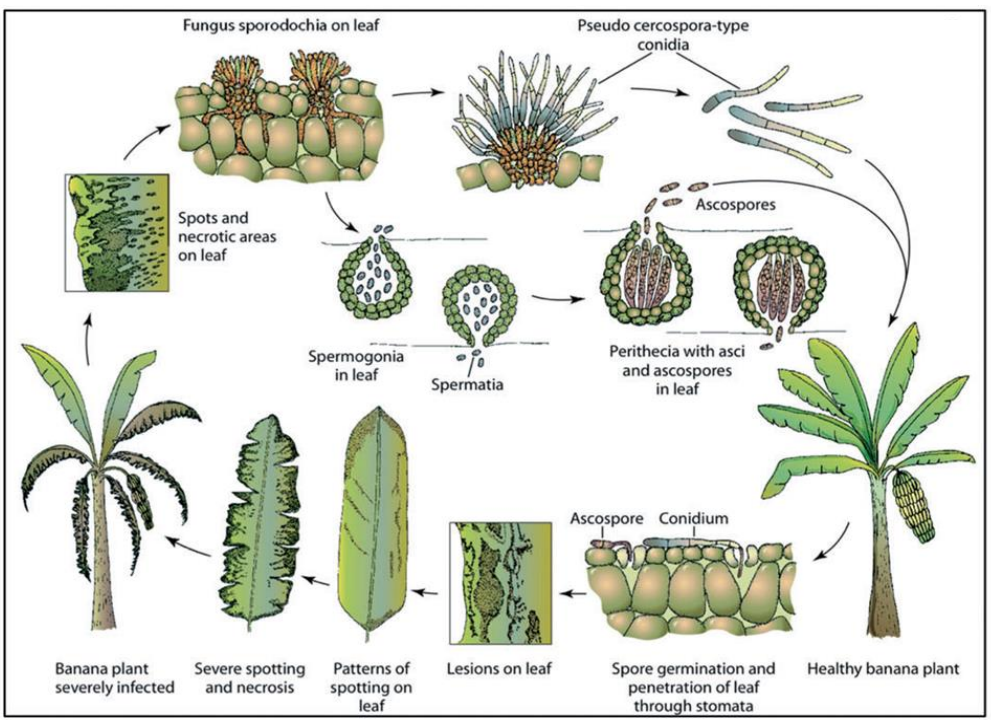

Figure 3. *Pseudocercospora fijiensis* life cycle reprinted from Agrios (2005) as shown in Churchill (2011).

The first conidiophores of *P. fijiensis* develop on the abaxial surface of the leaf during stage 2 or 3 and continue to develop until stage 5. Conidia production is almost continuous between stage 3 and stage 5. Conidiophores emerge individually or in diverging fascicles of two to eight stalks from the stomata of the abaxial surface of the leaf (within the boundary of the lesion). Few conidiophores emerge from stomata on the adaxial surface of the lesion (Meredith & Lawrence 1969). Conidia are pale greenish or olivaceous in colour and obclavate to cylindro-obclavate in form. Conidia contain one to 10 septae that form a straight or mildly curved propagule, with a short obconically truncate base. Conidia have a visible and slightly thickened hilum 1.5-3 µm of diameter and an obtuse tip, 20-132 µm long, of 2.5-5 µm diameter at the broadest point near to the base, tapering to 1.5-3 µm diameter at the tip (Meredith & Lawrence 1969).

The sexual reproduction starts with the development of spermogonia at stage 3 or 4 at the lower leaf surface. Spermogonia with spermatia become abundant at stage 4 (Meredith & Lawrence 1969). On the abaxial surface, spermogonia develop frequently in the substomatal cavity of stomata from which one or more conidiophores emerge. Mature spermogonia contain hyaline, rod-shape spermatia that act as gametes in fertilization of the pseudothecia that emerge in stage 5 and 6 (Meredith & Lawrence 1969). Pseudothecia are present at both sides of the lamina, but more frequent on the adaxial surface. The pseudothecia are scattered, immersed, piercing the epidermis by a narrow or moderately thick papillate ostiole, globose with 50-85 µm of diameter. Their wall is composed of three or more layers of dark brown polygonal cells and they contain numerous asci that are bitunicate, obclavate, and contain eight two-celled hyaline fusoid-clavate ascospores (dimensions 11.5-15.6 x 2.5-5.0 µm, Figure 4), with the larger cell uppermost in the ascus (Meredith & Lawrence 1969).

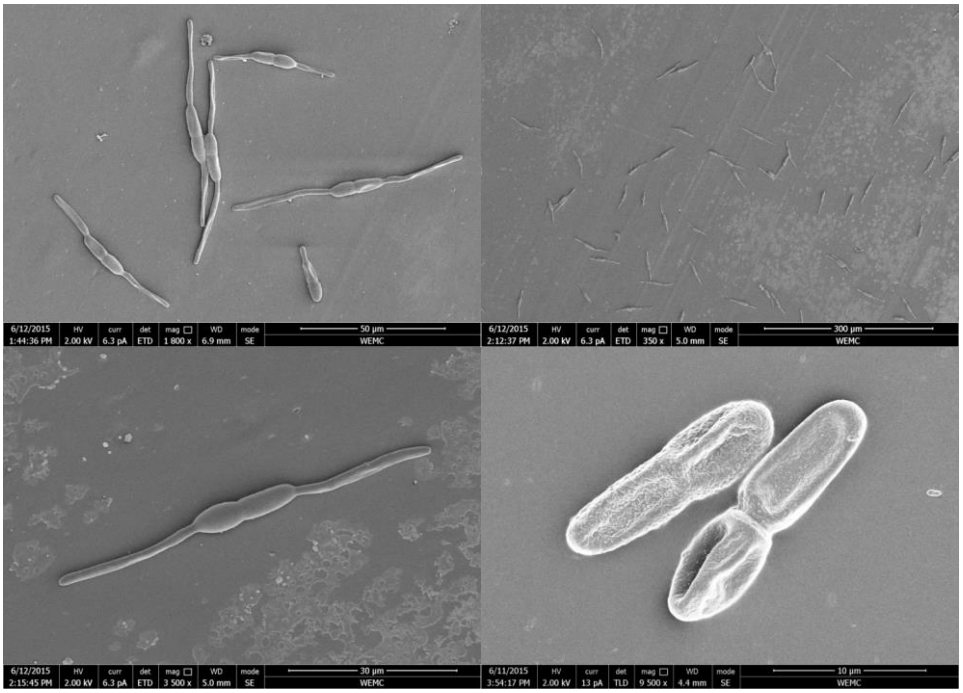

Figure 4. *Pseudocercospora fijiensis* ascospores in cryogenic Scanning Electron Microscope (CRY-SEM).

Pseudocercospora fijiensis genetics

Pseudocercospora fijiensis is a haploid, ascomycete fungus, belonging to the family *Mycosphaerellaceae,* order *Capnodiales,* class *Dothideomycetes,* and phylum *Ascomycota.* The fungus has an asexual and sexual reproduction cycle driven by a bipolar heterothallic mating system (Churchill 2011b). The dissemination of *P. fijiensis* most likely started in Southeast Asia and since then has spread to the major tropical and subtropical banana growing areas of the world (Churchill 2011b; Long 1979; Robert *et al.* 2012). This has been supported by a number of population genetic analyses that indicate Southeast Asia, between Papua New Guinea and the Solomon Islands (Carlier *et al.* 1996; Halkett *et al.* 2010; Hayden *et al.* 2003; Rivas *et al.* 2004b) as the centre of origin. Consequently, founder effects and bottleneck phenomena were detected in populations outside Southeast Asia, which are potentially the main determinants of

the global population structure (Carlier *et al.* 1996; Fahleson *et al.* 2009; Halkett *et al.* 2010; Manzo-Sanchez *et al.* 2008; Rivas *et al.* 2004b; Zandjanakou-Tachin *et al.* 2009). This is consistent with the stochastic nature of the disease spreading at continental and local scales in contrast to a steady advance of an epidemic front, although some populations within countries show a continuous range expansion as an epidemic front (Halkett *et al.* 2010).

The underlying mechanism for stochastic disease development at local or continental scale could result from either airborne spores or from the transport of infected plant material (Halkett *et al.* 2010; Rivas *et al.* 2004b; Zandjanakou-Tachin *et al.* 2009). Most of the *P. fijiensis* populations are not in gametic disequilibrium, resulting in high gene diversity levels. This supports the hypothesis that random-mating – in accord with a heterothallic bipolar mating system, similar to the related *Zymoseptoria tritici* (Conde-Ferraez *et al.* 2007; Goodwin & Kema 2014; Kema *et al.* 1996; Waalwijk *et al.* 2002) exists within the population and underlines the important role of ascospore dissemination (Carlier *et al.* 1996; Halkett *et al.* 2010; Rieux *et al.* 2014; Rivas *et al.* 2004b). The colonization patterns are also supported by genetic studies from a historical perspective. For example, the first report of *P. fijiensis* in Latin America was in Honduras and Costa Rica (Guzmán *et al.* 2013), which is congruent with the highest gene diversity levels found in these countries (Rivas *et al.* 2004b). In other Latin American countries the genetic diversity is considered medium to low in comparison with Costa Rica and Honduras (Perea *et al.* 2005). In Africa, the point of entry is more ambiguous since the genetic diversity levels are comparable throughout most countries (Fahleson *et al.* 2009; Rivas *et al.* 2004b; Zandjanakou-Tachin *et al.* 2009).

Black Sigatoka disease management relies on fungicides

As mentioned above, black Sigatoka management in banana is difficult as the majority of commercial banana varieties is highly susceptible to *P. fijiensis.* In contrast to a plethora of other crops, plant breeding in banana is also limited and hence, cultivars with improved resistance to *P. fijiensis* hardly enter the market. In any case, the Cavendish subgroup, which is a major constituent of the global banana production, is extremely susceptible to black Sigatoka. Therefore, disease control relies either entirely on fungicides or production moves to sub-optimal environments that slow down disease development, such as the high altitude desert area of Piura, Peru.

In the banana industry two groups of fungicides are applied to control *P. fijiensis*. These are categorized according to their phytomobility into the plant tissue and characterized as contact and systemic fungicides. Contact fungicides remain at the leaf surface. Systemic or penetrating fungicides on the other hand are able to penetrate the plant tissue. The most widely applied systemic fungicides for black Sigatoka control are those belonging to the chemical families benzimidazoles, triazoles, morpholines and strobilurins (Pérez 2006).

The benzimidazoles were the first modern site-specific penetrating fungicides used for fungi pathogen control (Latin 2011). This group includes benomyl and thiabendazole.

In the 1980s, triazole (azoles) fungicides were introduced. They belong to the Demethylation Inhibitor fungicides (DMIs) that obstruct the lanosterol 14α-demethylase which catalyses the removal of a methyl group from lanosterol. This is an essential enzyme of the ergosterol biosynthesis pathway (Cañas *et al.* 2009; Pietila *et al.* 2006). The lanosterol 14α-demethylase enzyme is encoded by the *cyp51* gene.

Morpholine fungicides are well known by the compound Tridemorph that was first approved in 1969 (Pérez 2006). Tridemorph inhibits the Δ8 – Δ7 isomerase and the C14 reductase in the ergosterol metabolic pathway.

The first strobilurin fungicide was introduced to the global market in 1997 (Knight *et al.* 2002). The quinol oxidation inhibitors (QoIs), or strobilurins, block the respiration pathway by inhibiting the cytochrome bc1 complex in mitochondria (Peres *et al.* 2014). By 2000, resistant *P. fijiensis* isolates emerged on banana production farms in Costa Rica (Amil *et al.* 2007).

The newest compounds introduced in the market are "second generation" carboxamides. These fungicides are classified by the Fungicide Resistance Action Committee (FRAC) as Succinate Dehydrogenase Inhibitors class or SDHIs. Their fungicidal activity is based on the disruption of the mitochondrial tricarboxylic acid cycle (TCA) through inhibition of the Succinate Dehydrogenase enzyme (SDH). At the molecular level, carboxamides inhibit ubiquinone reduction by binding to the ubiquinone binding site (Qp site) of the SDH enzyme (Scalliet *et al.* 2012).

Scope of the thesis

This study aims to elucidate the molecular mechanisms on adaptation to a major class of fungicides, the sterol demethylation-inhibitors (DMIs), that are globally used in the disease management of black Sigatoka caused by *P. fijiensis.*

Chapter 1 provides a general introduction to the subject with a description of the importance of banana as a food and fruit crop, the causal agent of black Sigatoka - *P. fijiensis* - and its lifestyle and the disease expression and management.

Chapter 2 describes the history of black Sigatoka disease management and the role of fungicides and the decline in efficacy with a focus on the situation in Costa Rica, which is exemplary for other important banana growing regions.

Chapter 3 contains a global phenotypic and genotypic analysis of *P. fijiensis* isolates to describe and understand reduced efficacy to DMI fungicides and the relationship with *Pfcyp51* gene and promoter modifications and associated CYP51 protein three-dimensional modifications.

Chapter 4 summarizes a functional analysis of the *Pfcyp51* gene and describes promoter swapping experiments between wild type and resistant *P. fijiensis* isolates to analyse the role of repeat elements - present in the *Pfcyp51* promoters of resistant *P. fijiensis* strains - in the expression of the gene and its relation to reduced efficacy.

Chapter 5 addresses the question whether the *Pfcyp51* gene is the only determining factor in reduced DMI efficacy by an unbiased mapping approach where two resistant isolates are crossed to a sensitive strain resulting in two *P. fijiensis* mapping populations that were genotyped and phenotyped for DMI sensitivity. In both populations, a causal genetic region of 250,660 bp is identified that contains 53 putative genes, including the *Pfcyp51*.

Chapter 6 discusses the experimental outcomes of the preceding chapters, puts these in a broad context and concludes that *Pfcyp51* gene and promoter modulation is largely responsible for the reduced DMI efficacy in black Sigatoka disease management. The impact and implications of these findings are discussed for the development of future sustainable disease control strategies.

Chapter 2

An historical treatise and critical review of black Sigatoka control in banana production

Pablo Chong, Claudiana Carr, Gilberth Murillo, Mauricio Guzmán, Jorge Sandoval and Gerrit H.J. Kema

Introduction

Bananas are among the most important crops worldwide (Ploetz 2000; Ploetz *et al.* 2015). The 2012 – 2013 banana market review from the Food and Agriculture Organization of the United Nations (FAO), stated that the global production of bananas was around 106.7 millions of tons. Global export reached 16.5 million tons with a gross production value of US $29.7 billion (FAO 2014a, b). Nonetheless, global export of banana represents only 15.5% of the total banana production. The remaining 84.5% represents banana production for local consumption. This stresses the importance of the banana fruit as a staple food in many tropical and subtropical developing countries. It is believed that banana is a starchy staple food for approximately 500 million people (Collins 2014). Many banana varieties for local consumption are relative cheap and easy to produce. Sadly, many of these varieties are susceptible to black Sigatoka disease, an important leaf defoliation disease that is caused by the dothideomycete fungus *Pseudocercospora fijiensis* (previously *Mycosphaerella fijiensis*) (Arango *et al.* 2016). The disease causes substantial direct and indirect losses due to defoliation and consequently reduced yields as well as due to premature ripening of the fruit, turning them into an unfit commodity for the export, respectively (Ploetz 2000). The main control measure involves frequent fungicide applications with a very high environmental and economic burden (Chong *et al.* 2016b; Chong *et al.* 2016c; Risède *et al.* 2010). As such, black Sigatoka has a major effect on subsistence production of banana and plantain since most of the smallholders are unable to afford the costs of these fungicides (Ploetz 2000). One of the major issues in black Sigatoka control has been the excessive and unplanned fungicide use in many banana farms worldwide (Lapeyre *et al.* 2010b). This uncurbed usage promoted resistance development within the pathogen population. Over time, resistance levels increased to such extent that the number of fungicide applications are now near maximum levels (Chong *et al.* 2016b). This can be well

illustrated by the history of the control of the disease in Costa Rica, which is one of the major banana export countries that had to deal with the disease soon after its incursion in Central America (Marín *et al.* 2003). The extent and well documented record of the disease management by the National Banana Corporation (CORBANA) enables a critical review of contemporary black Sigatoka management, further substantiating and underscoring the need for alternative disease management practices and strategic decisions towards sustainable and environmentally friendly banana production.

Black Sigatoka management through time

A historic picture of black Sigatoka management

Black Sigatoka disease was first report in Fiji in 1963 (Rhodes 1964) and arrived to Costa Rica in 1979 (Lapeyre *et al.* 2010b; Rivas *et al.* 2004b). When black Sigatoka arrived another pathogen *Pseudocercospora musae* (previously *Mycosphaerella musicola*), also known as yellow Sigatoka, was already present in the banana farms in Costa Rica (Stover 1962). Much of the strategies for the control for black Sigatoka disease have been adapted from the control of yellow Sigatoka. For example, the use of protectant fungicide begins with the arrival of *P. musae* to Central America in 1934. The United Fruit Company begins applying Bordeaux mixture ($CuSO_4$ + $Ca(OH)_2$) for the control (Stover, 1990). In 1941, Leach did a great deal when he identifies that the unfurling heart leaf ("candela or cigar") is the major target for the ascospores of *P. musae* and later *P. fijiensis.* He also probed that it is physically impossible for protectant fungicides to reach ascospores in the hart leaf since this leaf is constantly expanding and exposing under surface of the leaf cylinder. By 1946 Leach published his research on bananas leaf spot diseases in Gross Michel cultivar (Leach 1946). Another important control strategy, the use of oil sprays for the control, was discover by Guille and Guyot in 1956 (Guyot

& Guillé 1954; Stover 1990). Table 1 shows a time line history of the chemical control of the so call banana leaf spots diseases, yellow and black Sigatoka. Eventually, black Sigatoka displaced yellow Sigatoka in most of the banana production areas around the world becoming the omnipresent banana pathogen (Guzmán *et al.* 2013).

Since the 1950s the export banana trade has been dominated by the banana "Cavendish" varieties (D'Hont *et al.* 2012; Langhe *et al.* 2009), which are very susceptible to *P. fijiensis.* Hence, fungicides are major disease control agents in addition to a range of cultural practices. The latter includes the application of mineral oil, which has been practiced since the 1950s (Klein 1960)(Marin et al 2003) and slows down disease development (Pérez 2006; Stover 1990). Moreover, mineral oil became an important carrier for many fungicides. In many ways, mineral oil is a main component of conventional control, they protect and reduce the fungicide volume by forming a homogeneous mist that distributes active ingredients on the leaf, prevents evaporation and improves, in the case of many systemic fungicides, the penetration of the plant tissue.

Prior to 1970's, almost all fungicides were protectants (Ma & Michailides 2005). Ethylene bis-dithiocarbamates, such as maneb and mancozeb were introduced in the market in 1950 and 1958, respectively, and have been widely used for black Sigatoka control. Dithiocarbamates have a nonspecific mode of action (moa) with the thiol group blocking respiration and other important metabolic process (Gullino *et al.* 2010; Pérez 2006). Chlorothalonil is another compound that is still frequently used, only with water as oil mixtures are phytotoxic (Pérez 2006). It is a derivate of the phthalimides, which thiol group interferes with the glutathione pathway, A-coenzyme and 2-mercaptoethanol thereby reducing all metabolic activity of the fungal cell.

The benzimidazoles were the first modern site-specific fungicides for disease control (Latin 2011). They include benomyl and thiabendazole that are still being used for the control

of crown rot of the harvested and packed fruit. Benomyl, a methyl benzimidazole carbamate, was introduced in 1968 and inhibits fungal growth by binding to the β-tubulin thereby disrupting fungal cell division (Cañas *et al.* 2006). However, point mutations at positions 198 of the β-tubulin gene lead to one amino acid (aa) change, which provokes complete resistance in *P. fijiensis* populations without any apparent fitness penalty (Cañas *et al.* 2006; Pérez 2006).

The first triazole fungicide, propiconazole, was introduced for black Sigatoka control in 1984 (Stover 1990). It belongs to the group of sterol demethylation-inhibitors (DMIs) that inhibit the lanosterol 14α-demethylase (CYP51), an essential enzyme of the ergosterol biosynthesis pathway (Cañas *et al.* 2009). This enzyme catalyses the removal of a methyl group from lanosterol in fungi, allowing the sterol metabolism to proceed to the production of ergosterol. Triazoles inhibit the CYP51 function by binding to the heme cofactor in the active site of the enzyme. Henceforth, sterol metabolism is hampered due the accumulation of 14-α-methyl-3,6-diol, a toxic sterol produced by the Δ-5,6-desaturase encoded by the gene ERG3, which affect membrane integrity and leads to growth inhibition and cell death (Lupetti *et al.* 2002; Pietila *et al.* 2006; Shapiro *et al.* 2011). Several studies have shown a correlation between the loss of sensitivity to triazoles and point mutations in the *cyp51* gene (Cañas *et al.* 2009; Lepesheva & Waterman 2004). In *P. fijiensis* such mutations are abundant and resistance is also due to overexpression of the *Pfcyp51*, as a result of promoter insertions (Chong *et al.* 2010; Chong *et al.* 2016b; Díaz-Trujillo *et al.* 2016a)

The morpholine fungicides are represented by tridemorph that was approved for black Sigatoka control in 1969. It inhibits the Δ8–Δ7 isomerase and the C14 reductase in the ergosterol metabolic pathway and its translocation is essentially trans-laminar (Pérez 2006). Therefore, it has a limited risk of resistance development in the pathogen population. Despite this advantage, its efficacy for black Sigatoka control is not as good as the azole fungicides (Pérez 2006).

The first quinol oxidation inhibitor (QoI), or strobilurin was introduced in 1997 (Amil *et al.* 2007; Knight *et al.* 2002). It blocks the respiration pathway by interfering with the cytochrome bc1 complex in the mitochondria (Pérez 2006; Sierotzki *et al.* 2000). Due to their entirely new chemistry and enormous efficacy, strobilurins quickly became one of the most important agricultural fungicides accounting for >20% of the global fungicide market within the first ten years after their introduction (Fernández *et al.* 2010). However, by 2000, isolates of *P. fijiensis* with resistance to the QoIs were already common on some farms in the production zones of Costa Rica (Amil *et al.* 2007), and in 2008, *P. fijiensis* populations at three commercial plantations in Costa Rica were almost fixed for strobilurin resistance (Arango *et al.* 2016). The resistance to these compounds is mediated by a point mutation in the *Pfcytb* gene that leads to a change of a glycine for an alanine at position 143 (G143A) of the protein (Sierotzki *et al.* 2000).

Finally, the latest fungicides for black Sigatoka management that entered the market are the "second generation" carboxamides or succinate dehydrogenase inhibitors (SDHIs). First generation carboxamides were discovered in the mid 1960's for the control of basidiomycetes. Their fungicidal activity is based on the disruption of the mitochondrial tricarboxylic acid cycle (TCA) through inhibition of the succinate dehydrogenase (SDH) enzyme. At the molecular level, carboxamides inhibit ubiquinone reduction by binding to the ubiquinone binding site (Qp site) of the SDH enzyme (Scalliet *et al.* 2012). New molecules with a wider spectrum of activity were discovered recently (Leroux *et al.* 2010) but are not yet generally applied.

Table 1. Historical time line of the evolution and important events in the chemical control of black Sigatoka

Year	Black Sigatoka strategies evolution and important events	Reference
1934	Arrival of *Pseudocercospora musae* (*Mycosphaerella musicola)*, also called yellow Sigatoka, to Americas and the beginning of the use of Bordeaux mixture.	Stover 1962
1941	Leach identifies the unfurling cigar (heart) leaf as the major target for the *Pseudocercospora musae* spores. He also proves that the heart leaf cannot be protected by contact fungicides.	Leach 1946
1946	Leach publishes his discoveries in "Banana leaf spot (*Mycosphaerella musicola*) on the Gros Michel variety in Jamaica: "Investigations on the aetiology of the disease and the principles of control by spraying".	Leach 1946
1950	The first dithiocarbamate, Maneb, was introduced for yellow Sigatoka control.	Stover 1990
1956	Guille and Guyot discovered oil sprays.	Guyot & Guillé 1954
1958	Introduction of the dithiocarbamate mancozeb (Dithane) for the control of yellow Sigatoka. Maneb successfully controls leaf spot diseases together with copper applications by aircraft.	Stover 1990
1960	Klein publishes the first forecasting method of oil spray in Honduras. He shows that oil controls the streak disease stage and that the stages of the disease can be used as a decision moment for fungicide application.	Klein 1960
1962	Discovery that oil-in-water mixtures are more effective than oil alone.	Stover 1990
1963	First report of black Sigatoka in Fiji.	Rhodes 1964
1967	DuPont sent the first systemic fungicide (the first benzimidazole), compound number 1991 or Benlate (benomyl), to be evaluated in Honduras. In the same year Calixin (tridemorph) was also tested in Honduras.	Stover 1990
1972	• The second forecasting method was developed in Guadalupe by Ganry and Meyer, who divided the disease symptoms in five stages. They correlated disease development with temperature and evaporation rates. This was the first system introducing oil and systemic fungicides (Benlate). • Black Sigatoka is discovered in Honduras and from there it dispersed throughout Latin America and the Caribbean.	Ganry & Meyer 1972 Guzmán *et al* 2013
1973	First epidemic of black Sigatoka disease in Honduras. Benlate was effectively and extensively used for disease control. First use of in-oil mixtures and later in "cocktails" (different mode of action fungicide mixtures) with Mancozeb.	Stover 1990
1976	First fungicide resistance appeared against Benlate in Honduras, followed by a serious outbreak of black Sigatoka.	Stover 1990
1979	Chlorothalonil was introduced, but was unable to provide adequate control under conductive conditions for the disease (heavy rain promoting abundant ascospore production).	Stover 1990
1980	The Fungicide Resistance Action Committee (FRAC) was founded as an organization designed to discuss resistance problems and to formulate plans for cooperative efforts to avoid or manage fungicide resistance.	http://www.frac.info/about-frac/why-frac-
1981	Dithane, Benlate and Calixin were reintroduced to control black Sigatoka. At that time protectant fungicides were used combined with cocktails during rainy periods and almost exclusively in dry periods.	Stover 1990
1983	First report of black Sigatoka in Cameroon. The symptom development method was modified to six stages by Fouré.	Fouré 1985, Stover 1990
1984	The first triazole fungicide propiconazole belonging to the DMI group was introduced together with a forecasting method to control black Sigatoka.	Stover 1990

Year	Black Sigatoka strategies evolution and important events	Reference
1986	Flusilazole fungicide is introduced for black Sigatoka control. Propiconazole and flusilazole were extremely effective having curative effect when applied 14 days after the cigar leaf unfurled. Both compounds were extremely persistent in the leaf (up to 60 days after application) and were applied as oil-in-water emulsions. The decision supports system (forecasting) and the use of triazoles reduced the number of application cycles for black Sigatoka control from 35 - 45 to 20.	Stover 1990
1987	Guidelines were established to reduce the development of resistance in *Pseudocercospora fijiensis* to azoles and included no year-round applications (max. 4 - 8 cycles over six month).	Stover 1990
1988	Fungicide resistance monitoring methods were discussed/described during the first meeting on the use of DMI fungicides.	Stover 1990
1997	The first quinol oxidation inhibitor (QoI) or strobilurin was introduced.	Amil *et al* 2007
2000	Resistance to the QoIs already common on some farms in the productions zones of Costa Rica.	Amil *et al* 2007
2003	• Reduced efficacy of DMIs towards *P. fijiensis* is prevalent in many countries. • FRAC Banana Working Group is established.	Marín *et al* 2003 http://www.frac.info/about-frac/why-frac-
2004	Anilinopyrimidines are introduced.	Guzmán 2007
2006	DMI sensitivity is not "restored" after refraining from application over a period of four years in Costa Rica.	Guzmán
2008	*P. fijiensis* populations in Costa Rica are nearly fixed for QoI resistance (92-100%).	Arango *et al* 2016
2009	• Correlation between reduced efficacy of triazoles and point mutations in the *Pfcyp51* gene discovered. • Overexpression of the *Pfcyp51* gene is found in Costa Rican samples from 2009.	Cañas *et al* 2009 Chong et al 2012, Díaz et al 2016 (Chapter 3 and 4)
2010	• Irrespective of the country of origin in Latin America, the baseline sensitivity for boscalid, fluopyram and isopyrazam is high. • Black Sigatoka incursion in Martinique.	http://www.frac.info/docs/default-source/working-groups/banana-group/group/2010-meeting-minutes---english.pdf?sfvrsn=4 Ioos *et al* 2011
2012	Black Sigatoka incursion in Guadalupe.	Guzmán *et al* 2013
2016	First genetic mapping of DMI resistance in *P. fijiensis*.	Chong et al 2016c (Chapter 5)

DMIs as major control agents for black Sigatoka management

Currently, the DMIs are the major control agents for black Sigatoka in banana. The *P. fijiensis* DMI baseline sensitivity in Costa Rica has been continuously rising: In 2003, Marín et al., reported an average EC_{50} for propiconazole of 0.15 $mg.L^{-1}$ with a maximum value of 0.5 $mg.L^{-1}$ in Costa Rica populations (Marín *et al.* 2003). Díaz et al., (2016) showed an increase

among four resistant Costa Rican isolates sampled in 2009 to an average of 1.10 $mg.L^{-1}$ with a maximum value of 1.53 $mg.L^{-1}$ and recently Chong et al. (2016) determined an EC_{50} average of 5.8 $mg.L^{-1}$ and a maximum value of 18.4 $mg.L^{-1}$ among 107 *P. fijiensis* isolates from 2014 (Chong *et al.* 2016b). This shows that the EC_{50} between 2003 and 2014 gradually increased with an average of 1.4 fold per year. For example, based on 2009 the measured average was 1.1 $mg.L^{-1}$ and predicted by calculation, is 1.12 $mg.L^{-1}$. Therefore, the increment is predictable and it is currently 38 times higher than in 2003. Evidently, one can argue that the locations and sample numbers need to be considered but the sensitivity shift is clear.

As mentioned above, Costa Rica has a long history of black Sigatoka control with a high number of fungicides applications per year (Lapeyre *et al.* 2010a; Marín *et al.* 2003). The observed DMI baseline sensitivity shift correlates with the increasing amounts of applied fungicides, which increased from 30 in the 1990's up to 50 treatments by 2007 and up to 53 in 2015 on the San Pablo plantation (Figure 1). In a global survey for DMI efficacy, Chong et al., (2016) recently reported that EC_{50} values among Costa Rican *P. fijiensis* isolates are representative for the selective pressure exerted by DMIs fungicides on the pathogen population. Interestingly, at least for the case of "San Pablo", the actual number of DMI cycles reduced over time from seven to four applications per year in 1998 and 2014, respectively, but overall the number of DMI cycles was approximately 10 between 2003 and 2010. From 2014 to 2015, there was a sudden rise in the number of DMI cycles (from four to seven; Figure 2) that maybe a consequence of the frequency of less sensitive isolates with high EC_{50} values in the "San Pablo" population (sample taken in 2014, Figure 3) (Chong *et al.* 2016b). This suggests that the selective pressure in previous years was sufficient to turn the major part of the population into resistant strains by 2014.

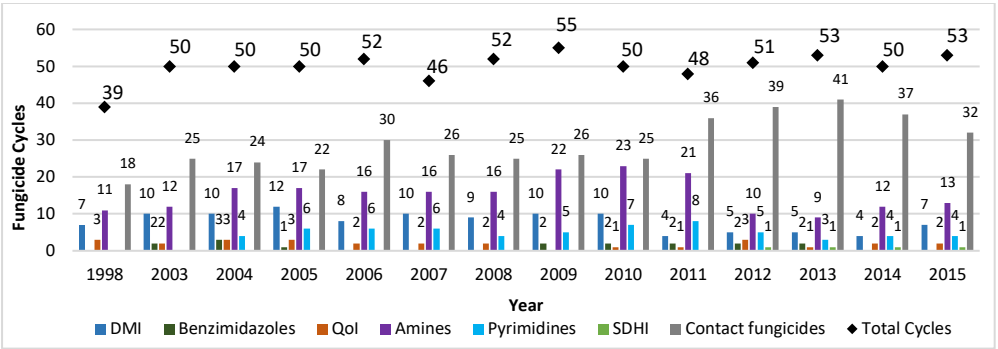

Figure 1. An overview of different chemicals used to control black Sigatoka at the San Pablo plantation in Limon, Costa Rica, in 1998 and during the period 2003-2015. Numbers over the coloured bars indicate the annual frequency of applications per chemical group. The numbered diamonds over the bars indicate the total number of fungicide cycles per year (One cycle can include several fungicides in mixtures).

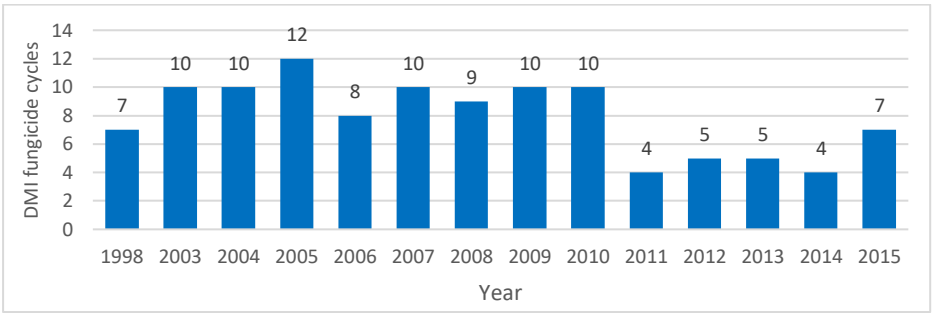

Figure 2. An overview of the number of DMI applications for the control black Sigatoka at the San Pablo plantation in Limon, Costa Rica, in 1998 and during the period 2003-2015.

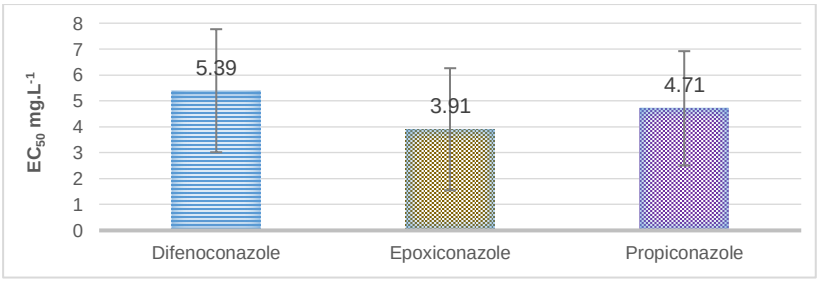

Figure 3. The average sensitivity, measured as EC_{50}, of 49 randomly collected mono-ascosporic *Pseudocercospora fijiensis* strains from the San Pablo - Limon, Costa Rica - population to three DMI fungicides in 2014. Strains with an EC_{50} values >1 $mg.L^{-1}$ are considered resistant (Chapter 3) (Chong *et al.* 2016b).

It is important to mention that the aforementioned reduction of DMI cycles at "San Pablo" between 2011 to 2014 was driven by forecast information (weather and disease development) and economic considerations, but not by the reduced efficacy of the compounds. In addition, ceasing DMI applications for a period of four years hardly changed the frequency of resistant strains in the population. This suggests no fitness penalty for reverting to sensitivity. Previous studies in *P. fijiensis* have not shown significant differences in incubation period, latency period, conidial sporulation and severity between DMI sensitive and resistant isolates (Romero 1999). Hence, the use of DMI free periods will likely not contribute to population shifts towards sensitivity. On the contrary, by the end of the four years without DMIs fungicides the frequency of DMI resistant strains increased in both treatments (Figure 4), which might, however, also result from the immigration/gene flow from the DMI experimental plots (plots of 50 ha with 200 ha borders between experimental plots) since the airborne *P. fijiensis* ascospores can travel at least hundreds of meters (Guzmán 2007; Lapeyre *et al.* 2010b; Rieux *et al.* 2014). The increased use of mancozeb, azoxystrobin and tridemorph compensated for reduced cycles with DMIs, but exerted – of course – significant selective pressure for these fungicides (azoxystrobin and tridemorph), and has resulted in *P. fijiensis* populations that are nearly fixed for strobilurin resistance, surely compared to the wild type (wt) population of San Carlos (Arango et al., 2016).

The historical records (2001 – 2015) for DMI sensitivity monitoring (discriminatory dose 0.1 $mg.L^{-1}$) of ascospores germ tube lengths from different Costa Rican banana regions enable a comparison of wt site San Carlos with commercial banana plantations (Figure 5). In general, the loss of sensitivity curve fluctuates, but seems to stabilize in the last 5 years, which can be explained by the implementation of better control (with mixtures of different target-site specific and protectant fungicides) and management strategies during recent years.

Nonetheless, germ tube lengths monitoring experiments have unequivocally shown the reduced efficacy of DMIs for Costa Rican *P. fijiensis* populations (Chong *et al.* 2016b).

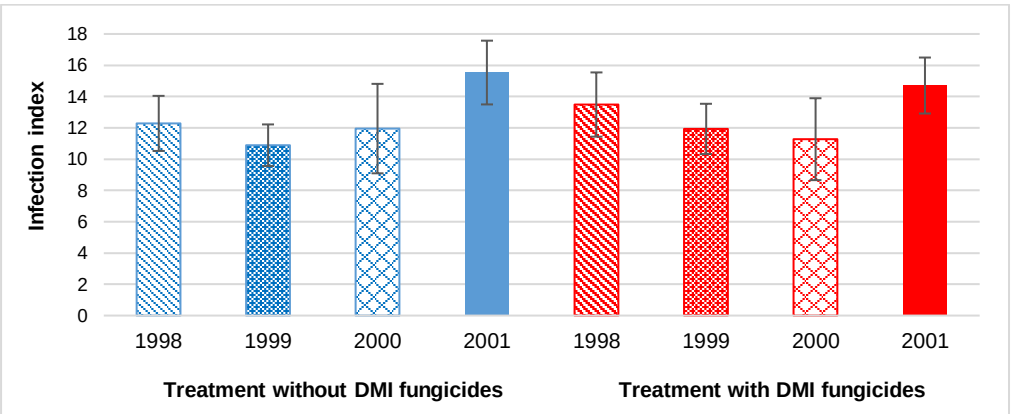

Figure 4. The infection index of *Pseudocercospora fijiensis* on Cavendish banana at the San Pablo plantation – Limón, Costa Rica - in plots that were either treated with DMIs or that underwent alternative treatments without DMIs from 1998 to 2001 (Guzmán 2007).

Evidently, it is possible that the contrast in sensitivity levels is determined by the two monitoring procedures - EC_{50} based on mycelial growth in 96-well plates vs. ascospores germ tube lengths - or by the physiological differences of the tested tissues (mycelium vs. ascospores). For example, ascospore monitoring procedures are evaluated with two or three doses at 48 hours with a considerable number of samples (on average 100 spores per treatment with more than three repetitions). However, only three doses preclude observing accurate response levels, despite the representative number of ascospores per population. Alternatively, some fungicidal effects might be expressed at stages after 48 hours. Calvo et al. (1997) showed that the germ tube of propiconazole resistant ascospores continued growing after 48 hours, while those of some sensitive ascospores stopped growing after 48 hours (Calvo *et al.* 1997). In contrast, mono-ascosporic colonies are evaluated with eight different doses and after five to

10 days incubation with mycelium pieces in 96-well microtiter tests (Chong *et al.* 2016b), a method that is very common for other fungi, such as the related *Zymoseptoria tritici* where thousands of isolates are monitored on an annual basis by various laboratories (http://www.frac.info/monitoring-methods). Hence, this method is more precise to determine overall sensitivity of an individual strain, but requires a range of preliminary experiments for standardization and statistical analyses as well as sufficient samples to represent a given population. Despite the different levels, however, the shift of base line sensitivity is recorded independent of these two methodologies, which is also apparent by analysing the *P. fijiensis* populations in commercial farms with the wt population at San Carlos (Figure 6; Chong et al., 2016). In the commercial banana farms the percentage of inhibited ascospores tends to decrease. Ascospore germ tubes inhibition in the range of 50-70% (Figure 6) might result individuals with moderate sensitivity to DMIs or to tolerance, as defined by normal development despite substantial abiotic stress. Interestingly, we noticed that there is always a very low percentage of isolates from the control population with high levels of DMI resistance as well as very sensitive ascospores from overall resistant populations at commercial farms. This supports the hypothesis that there are always sensitive isolates that escape disease control or resistant isolates that are able to survive and reproduce in a non-selective environment (Latin 2011; Vincellin 2014). We have not observed such blurred classes for strobilurin resistance in the same populations (Arango et al., 2016).

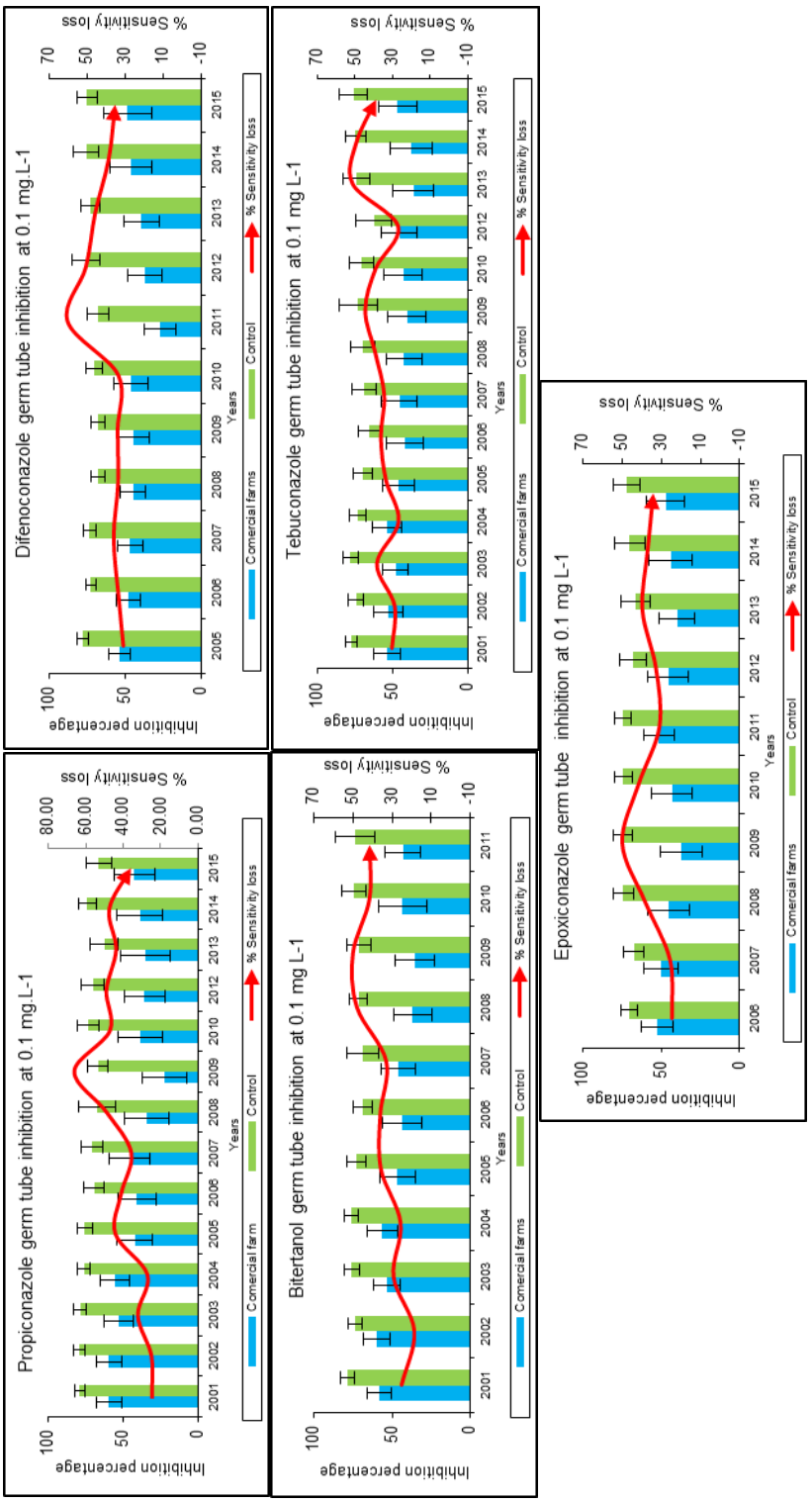

Figure 5. The inhibition of *Pseudocercospora fijiensis* ascospore germ-tube lengths at 0.1 mg.L^{-1} as a measure of sensitivity to the DMI fungicides propiconazole, difenoconazole, tebuconazole, bitertanol and epoxiconazole in control sites at San Carlos, Costa Rica and commercial Cavendish plantations in the main producing area in Limón. The red lines represent the trend of the sensitivity loss. Ascospore populations are considered resistant when the germination percentage is >70%, whereas as they range between 50% and 70% they are considered tolerant and once germination is <50% they are supposed to be sensitive to DMI fungicides.

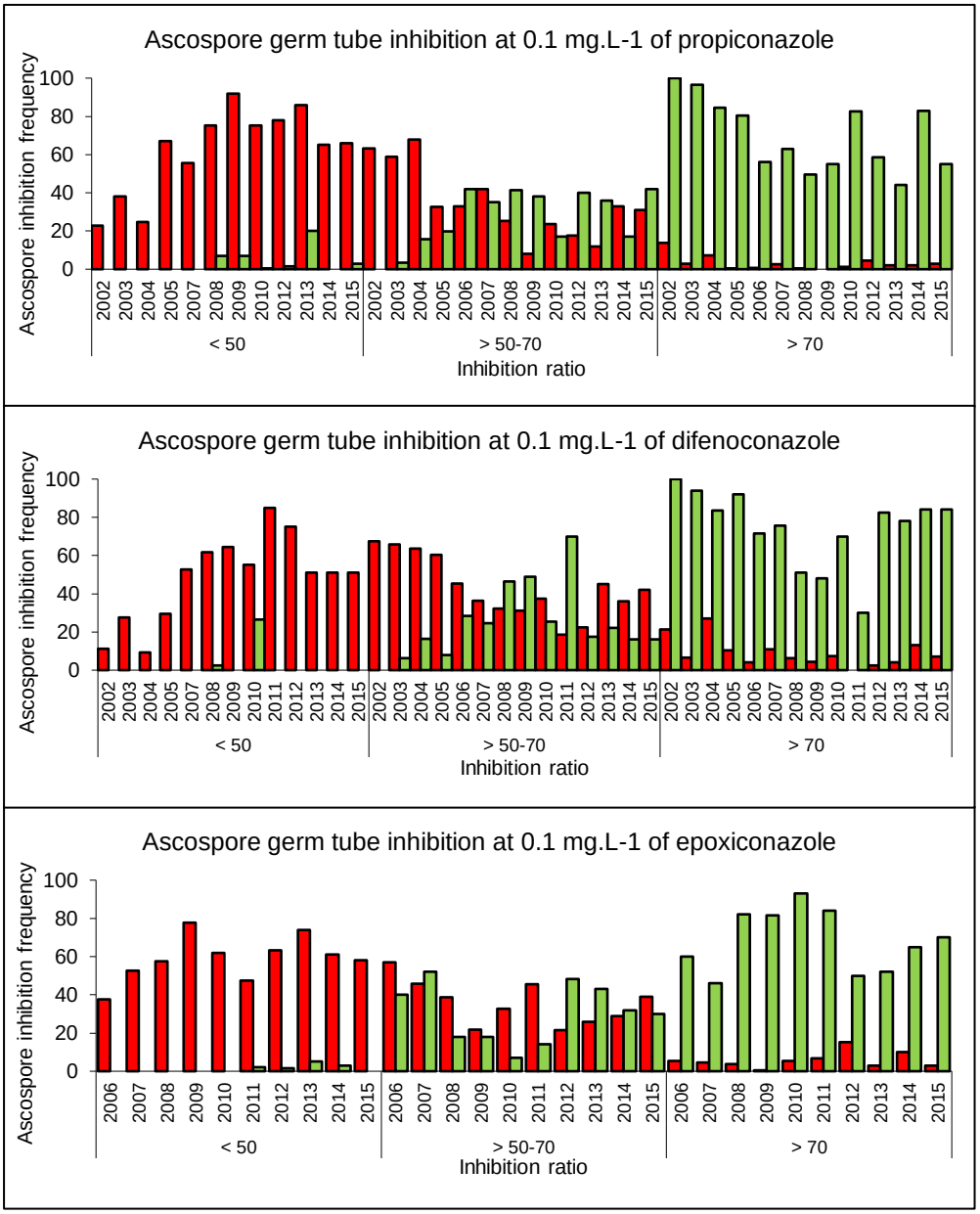

Figure 6. Comparison of the inhibition of *Pseudocercospora fijiensis* ascospore germ-tube lengths at 0.1 mg.L^{-1} as a measure of sensitivity to the DMI fungicides propiconazole, difenoconazole and epoxiconazole in either control sites at commercial Cavendish plantations in the main producing area in Limón (red bars) and San Carlos (green bars) Costa Rica. Populations with >70% inhibited ascospores are considered as sensitive, whereas those with inhibition percentage between 50% and 70% are supposed to be tolerant and <50% inhibited ascospores populations are presumably resistant.

Clearly, one of the most important questions about the selection pressure that DMI fungicide exerts on *P. fijiensis* populations is about the actual doses that reach the fungus. The translation from laboratory efficacy trails to field conditions is difficult and hardly tested. However, we have performed inoculation trails with *P. fijiensis* isolates of various resistance levels – as determined in the laboratory - and showed that some resistant strains were equally fit in greenhouse trials using field doses of DMIs (DMI doses of 400 $mg.L^{-1}$ Figure 7).

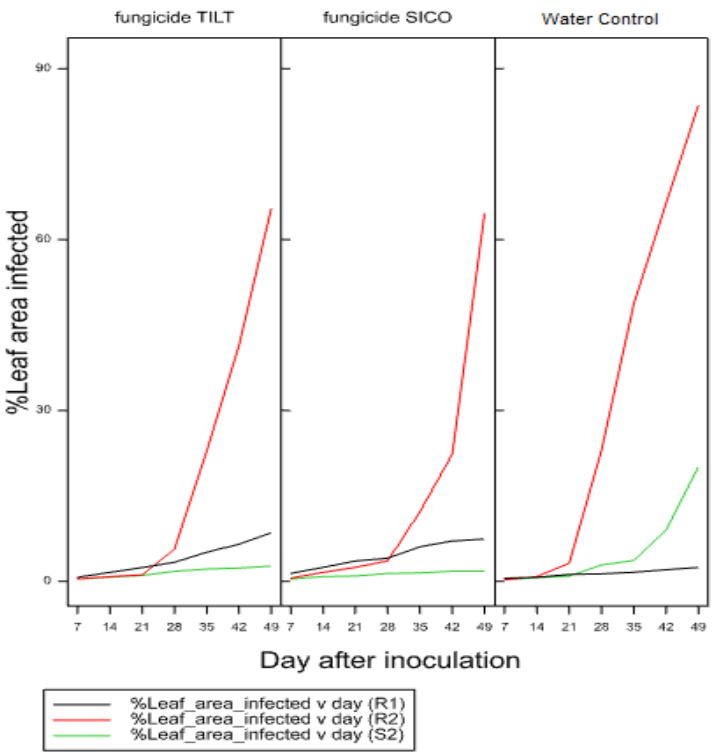

Figure 7. Phenotyping DMI sensitive and resistant *Pseudocercospora fijiensis* strains on Cavendish (var. Grand Naine) under greenhouse conditions. Plants were treated prior inoculation with two DMIs (propiconazole, Tilt, and difenoconazole, Sico) or with the water control (no fungicide application). Plots show a rapid development of infected leaf area by the resistant strain R2. Resistant strain R1 developed much slower but caused significantly more disease than the sensitive control (S2), Disease development was monitored between 7 and 49 days after inoculation (dai).

In general, most farmers follow the technical instructions of the manufacturers as printed on the label of the commercial fungicides, but the effective dose also depends on the "carrier", mineral oil or oil in water emulsions with different emulsifiers. Fischer (1991) reported that a mix of propiconazole in emulsion has a foliar level recovery of 90% compared to just 43% in mineral oil, although the latter had a significantly better leaf coverage than emulsions, particularly under moderate dew conditions.

Details on final DMI doses under field conditions are available in the – rare - Ciba-Geigy Tilt technical dossier from 1991. It provides one of the most complete descriptions of the application, leaf penetration and inside leaf degradation of the product. This important information should be available for each fungicide that is commercialized for black Sigatoka control. For example, oil in water emulsion was the best combination for leaf penetration from 13 $mg.L^{-1}$ of fungicide inside the leaf at the first hour down to a final dose of 2 $mg.L^{-1}$ at 96 hours after application. However, the most stable application was the mix with oil that despite a relatively low leaf penetration - at the first hour after application 5 $mg.L^{-1}$ – maintained a concentration of 6 $mg.L^{-1}$ for the next 11 hours and finally of 4 $mg.L^{-1}$ between 24 and 96 hours. Finally, a water formulation showed a penetration of 6 $mg.L^{-1}$ at the first hour, which dropped to only 0.5 $mg.L^{-1}$ at 96 hours. In general, the major fungicide part that penetrated the lamina disappears within 12 hours (biological degradation, dilution), but a residual low level of the active ingredient remains in the tissue for more than four days (Ciba-Geigy 1991). Still, it is worth mentioning that doses on the leaf are usually 1 to 1.5 times higher during the first 12 hours (the persistence of the fungicide on the leaf highly depends of the type of carrier) (Ciba-Geigy 1991). Hence, considering these results, the actual propiconazole doses that most pathogen spores and colonies face will be 1-5 $mg.L^{-1}$ at least for some days (Ciba-Geigy 1991), provided an appropriate distribution on/in the leaf. We, therefore, conclude that the empiric

dose of 1 mg.L^{-1} as a threshold for propiconazole resistance in laboratory efficacy trials has a solid support from leaf penetration experiments.

Cultural practices to reduce black Sigatoka impact

The control of the critical levels of black Sigatoka in banana plantations is driven by reducing direct costs- such as reduced yield potential – and indirect costs, due to reduced quality. The latter is far more important as the disease triggers early ripening, which makes the fruit unfit for export (Ploetz *et al.* 2015). Hence, the extraordinary control measures are required to deliver a marketable product that survives the demanding logistic chain (Guzmán *et al.* 2013; Ploetz *et al.* 2015). The favourable weather conditions for black Sigatoka development include high relative humidity (95 – 100%) with temperatures around 27°C and intermittent rain fall (Long 1979; Marín *et al.* 2003). The common agronomic factors that affect disease development include production site selection, banana variety selection, planting date, soil fertility and acidity, plant spacing, irrigation practices and field drainage (Vincellin 2014). As mentioned above, the underlying issues of black Sigatoka management is the fact that 95% of the export trade comprises highly susceptible "Cavendish" clones that essentially form one big monoculture around the globe (Ploetz *et al.* 2015). Hence, the reduction of on-farm inoculum is one of the most important cultural practices, which can be achieved by removing leaf tissue with mature pseudothecia that release ascospores. This practice is known as "deleafing", "detipping" or "surgery" (Marín *et al.* 2003). The detached foliage will rapidly decompose on the plantation floor, but still may provide inoculum. Therefore, decomposition is stimulated by adding products, such as urea, which altogether can reduce the infectiousness by 50% (Marín *et al.* 2003). Water logging is a potential threat to banana plantations under tropical conditions and, hence, appropriate drainage, plant spacing or drip-irrigation are crucial for optimal plant

development and to reduce excessive humidity that supports disease development (Marín *et al.* 2003).

The commonest fungicides and spraying programs for black Sigatoka management

Overall, the international banana industry just uses two kinds of fungicides for black Sigatoka management that are categorized according to their phytomobility, either contact or penetrant fungicides (Table 2). Contact fungicides remain on the surface of the leaf and they are only redistributed by precipitation, irrigation and dew. Penetrant fungicides, on the other hand are absorbed into the plant tissue and can be subdivided into acropetal, local penetrant or systemic penetrants (Latin 2011). In acropetal penetration compounds are moving between cells along with the water potential gradient. They are xylem mobile and therefore translocated upward towards the leaf tips and margins. Local penetrants diffuse into the wax/cuticle layer where they are bound and immobilized. Systemic penetrants move inter- and intracellularly with the live protoplast and follow a sugar density gradient (Latin 2011). The main contemporary compounds currently used for black Sigatoka management are contact fungicides such as mancozeb and chlorothalonil, and the acropetal penetrant fungicides such as the DMIs propiconazole, epoxiconazole, difenoconazole and tebuconazole. Despite the overall resistance development strobilurins such as azoxystrobin, pyraclostrobin and trifloxystrobin are still in use, likely due to their "greening effect" (Bennett & Arneson 2003; Pérez 2006). Finally, tridemorph (amine) has been also very important for the control of black Sigatoka, particularly in mixtures with protectants or systemic fungicides. Table 2 describes all the fungicides that have been recommended by the Fungicide Resistance Action Committee (FRAC) for the control of black Sigatoka. Usually, several fungicides are prepared as "cocktails" that are

composed of two or three fungicides with different modes of action. Generally, a mix is composed by one or two systemic fungicides and a protectant, for instance a DMI, an amine and mancozeb. Occasionally, QoIs (depending of the level of resistance in the population) or SDHIs are used instead of DMIs. QoIs are most of the time mix with amines or pyrimidines. Due to the epidemiology of the disease, with an almost continuum of ascospore production (Lapeyre *et al.* 2010b; Marín *et al.* 2003), contact fungicides remain a very important component of the tank mixes, sometimes combined with new or specialty products such as vegetable wax and various biologicals (Table S1).

At present, weekly spray schedules are required with around four applications per month (depending of the rain fall). This is significant more than e.g. the San Pablo applications schedule in 1998 that comprised three applications per month in general and two applications from May to August. At that time, most fungicides were applied alone and systemic fungicides were applied in a six-month period, alternating weekly with another moa or contact fungicide. In 2003, applications were raised to five per month in some periods and most fungicides were mixed in "cocktails"; either systemic/protectant or protectant/protectant tank mixtures, although these protectants were also applied alone. Then, from 2008 onwards, spray schedules were more or less the same with four applications per months using systemic fungicides applied in mixtures and alternating moa's over a six-month period. The number of applications was increased to five per month in 2009 which is unaltered since then. A summary of the application cycles and the specific systemic groups being applied is shown in Figure 1. Table S1 provides the actual San Pablo fungicide application schedule during 2015.

Table 2. Fungicides used in black Sigatoka control ordered by their chemical classifications (FRAC 2013, 2015).

Chemical class		Fungicide use in banana	Fungicide trade name*	Description
Contact fungicides	Benzene derivatives	chlorothalonil	Bravo, Bronco, Daconil	This compound interferes with the glutathione pathway, a coenzyme and 2-mercaptoethanol reducing thiol-group based metabolism in the cell.
	Carbamates	Mancozeb	Dithane	Mancozeb is placed in the subclass of carbamate pesticides called dithiocarbamates. As a cholinesterase inhibitor, it affects the nervous system.
Systemic fungicides	Demethylation inhibitors (DMI)	bitertanol	Baycor	These compounds inhibit the lanosterol 14α-demethylase, an essential enzyme of the ergosterol biosynthesis pathway.
		difenoconazole	Score, Sico	
		epoxiconazole	Opus	
		fenbuconazole	Indar	
		myclobutanil	Rally, Sisthane	
		propiconazole	Tilt, Bumper	
		tebuconazole	Silvacur	
		tetraconazole	Eminente	
		triadimenol	Bulldock, Caporal,	
	Amine fungicides	spiroxamine	Impulse	Amines are ergosterol synthesis inhibitions. Tridemorph inhibits the Δ8 – Δ7 isomerase and the C14 reductase in the ergosterol metabolic pathway.
		fenpropimorph	Volley	
		fenpropidin	Seeker 750	
		tridemorph	Calixin, Musaclean	
	Qo inhibitors (QoI)	azoxystrobin	Amistar, Bankit	Quinol oxidation inhibitors (QoIs) or strobilurins, block the respiration pathway by inhibiting the cytochrome bc1 complex in mitochondria.
		pyraclostrobin	Comet	
		trifloxystrobin	Tega	
	Anilinopyrimidines (AP)	pyrimethanil	Siganex	AP's inhibit the methionine biosynthesis. It should only be used in mixtures and in full alternation. To reduce selection pressure, the total number of applications is limited to eight per year and these should not represent more than 50% of total number of sprays.
	Benzimidazoles (BCM)	benomyl	Benlate	Fungicides with high systemic and curative activity that allow long intervals between applications. Resistant *P. fijiensis* strains were detected two years after first application.
		carbendazim	Curacarb	
		thiophanate	Cycosin	
		thiabendazole	Mertect	
		thiophanate-methyl	Nucilate, Thiophol, Topsin.	
	N-Phenylcarbamates	dietofencarb,	Powmyl	No sensitivity data are yet available. A maximum of 33% of the total number of sprays can be applied with N-Phenylcarbamates.
	SDHI fungicides	boscalid	Cumora	Irrespective of the country of origin in Latin America, the baseline sensitivity for boscalid, fluopyram and isopyrazam is high.
		fluopyram	Luna	
		isopyrazam	Reflect	
	Guanidines	dodine	Syllit	Irrespective of the country of origin in Latin America, the baseline sensitivity for dodine is variable. However, in Ecuador baseline sensitivity did not significantly change after two years of applications.

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

Molecular analyses of fungicide resistance in *P. fijiensis*

One of the main adaptations to environmental changes or selection pressure is the genetic variation of the target organism, which modulates and complicates sustainable disease control. Site-specific compounds often select for total resistance due to point mutations - often referred as “major gene” resistance or “monogenic” resistance - that renders these compounds ineffective (Latin 2011), including the mechanisms for resistance to benzimidazoles and strobilurins. Eventually, mutant alleles will dissipate in the population conferring partial or total resistance to a particular fungicide (Grünwald *et al.* 2003), whereby the epidemiology of the organism can amplify the effect and rate of dissemination (Aouini et al., 2016). The target of DMIs is lanosterol 14α-demethylase that is encoded by *Pfcyp51* (Cañas *et al.* 2009). Recent studies revealed a correlation between propiconazole resistance and point mutations in the *Pfcyp51* gene (Cañas *et al.* 2009; Chong *et al.* 2010; Lepesheva & Waterman 2004). The effect of point mutations in the *Pfcyp51* gene was also related with cross resistance to epoxiconazole and difenoconazole (Chong *et al.* 2016b). The plethora of *Pfcyp51* mutations has resulted in a total of 28 aa substitutions (Chong *et al.* 2016b). From this 28 aa substitutions, positions 136, 313, 380, 381 and 460 – 463 have been associated with loss of sensibility to DMI (Cañas *et al.* 2009; Chong *et al.* 2016b). These amino acid changes are nearby central positions of the lanosterol 14α-demethylase, surrounding the Substrate Recognition Site (SRS) (e.g. positions Y136, A313, 381) and inside a loop close to the L α helix (e.g. Y460 to Y463) (Cañas *et al.* 2009; Chong *et al.* 2016b; Lepesheva & Waterman 2004). The large variation in genetic isoforms complicates the analysis of the enzyme and the corresponding degrees of resistance. *Pfcyp51* promoter insertions were recently discovered as a driving mechanism for *Pfcyp51* expression contributing to quantitative variation for reduced DMI sensitivity (Chong et al., 2016a, 2016b; Diaz et al., 2016). Similar mechanisms were identified in *Aspergillus fumigatus* isolates that are resistant to medical azole fungicides (Mellado *et al.* 2007; Snelders *et al.* 2012;

Verweij *et al.* 2013). Interestingly, none of the DMI sensitive strains found in a global survey contained promoter insertions, while they were very common in tolerant and resistant strains and correlated with the levels of resistance to DMIs (Chong *et al.* 2016b). Overexpression of *cyp51* also correlated with promoter insertions in *Venturia inaequalis* and *Blumeriella jaapii* (Schnabel and Jones 2000, Ma et al. 2006), but their frequency in *P. fijiensis* is unparalleled. In *B. jaapii*, the overexpression results from upstream insertions of various truncated derivatives of LINE-like retrotransposons (Ma *et al.* 2006). However, the underlying mechanism and function of these repeated elements remains to be deciphered (Schnabel and Jones 2000, Ma et al. 2006; Diaz et al., 2016) and might involve blocking proper binding of expression reducing components or generate binding sites for positive regulators that enhance the promoter.

Supervised black Sigatoka control aided by disease forecasting systems

Forecasting systems have been essential tools for the control of black Sigatoka by using climatic and biological descriptors for the prediction of the severity of the disease (Guzmán 2007; Lapeyre *et al.* 2010b; Marín *et al.* 2003). These severity predictions are used for the timely application of the fungicide (Guzmán 2007; Lapeyre *et al.* 2010b). This allows a supervised rather than a calendar driven application, which supports efficient use of fungicides and modulates their application depending on necessary doses or frequencies. As mention above, most of the control strategies in black Sigatoka have been adapted from control programs that were developed for the milder yellow Sigatoka, which is caused by the fungus *P. musae*. The first forecasting for oil sprays was determined by symptom severity of controls that were only treated with oil (Stover 1990). Later, weather variables, such as temperature and evaporation rates, were additionally used to optimize fungicide applications by Ganry and Meyer (1972), including the use of oil and systemic fungicides (benomyl) and using a 0-5 scale

for symptom severity classes for yellow Sigatoka (Ganry & Meyer 1972; Stover 1990). After the incursion of black Sigatoka in Cameroon in 1983 the symptom development score was extended by Fouré with an additional severity class (stage 6) (Fouré 1985; Stover 1990).

Despite the overall success of these forecasting programs in a more supervised control of black Sigatoka for many years, they never took into account the cost of fungicide resistance. Since they only can be used with highly curative systemic fungicides (single target) the development of fungicide resistance interfered with their efficacy, leading to progressive abandoning of this rational strategy in favour of the systematic use of contact fungicides that must be applied every week (Lapeyre *et al.* 2010b). Hence, notwithstanding the fact that forecasting programs are still being used – in oil mixtures and based on different chemistries - as a decision making tool, there is a need for optimized and modernized programs to further fungicide efficiency in black Sigatoka control (Lapeyre *et al.* 2010b).

The way forward: integrating molecular DMI resistance parameters in disease management

Biological parameters such as the 'Stage of Evolution of Disease' (SED), the 'Youngest Leaf bearing Streaks' (YLSt), The 'Youngest Leaf Spotted' (YLS) and the 'Number of Functional Leaves at Harvest' (NLH) are very important for decision making and the evaluation of the efficacy of control strategies (Lapeyre *et al.* 2010b). Sensitivity monitoring procedures of the ascospores germ-tube inhibition have also been use for decision making, especially for the evaluation of the efficacy of control. Nonetheless, the information retrieved by this methodology is too superficial. Since is not possible to recover any molecular information from this method the information about the mechanism behind the resistance is lost. A more professional methodology has to be implemented integrating the molecular

information to understand the origin of the resistance. The currently available molecular information on modulation of the *Pfcyp51* gene, which seems the main driver for reduced sensitivity (Chong et al., 2016c), is a potential add-on for optimizing forecasting programs and hence, disease control. Quantitative information on overall *P. fijiensis* population characteristics (EC_{50} values, number of promoter inserts that can be monitored by simple PCR, but also cross resistance, multi drug resistance, fitness and virulence) in the target and neighbouring plantations - immigration and gene flow - can be used to predict the success of spraying cycles. The costs of generating these data is substantial due to sample/material preparation, but it provides a much broader view on the evolution of *P. fijiensis* populations that can be monitored and used to alert potential problems with reduced efficacy and hence inherent and increasing direct and indirect costs. With the continuously reducing costs for genome-based information, the use of this type of detailed information will positively contribute to optimize disease control. Governments and research institutes need to prepare themselves for advancing black Sigatoka management through interdisciplinary approaches using the latest technologies and alternative products to diversify and innovate control strategies. It is rather disturbing that monitoring of fungicide sensitivity in e.g. wheat is highly professionalized and entirely sequence based, but that black Sigatoka management is still using old-fashioned worn-out procedures that do not provide any insight in underlying mechanisms and thus prevent modernizing and rationalizing disease control. For instance, the lack of cross resistance between pyraclostrobin and azoxystrobin in *P. fijiensis* is nicely supported by a simple PCR test with specific molecular markers for the G143A substitution in the *Pfcytb* gene (Sepúlveda & Torres 2016). Such a quick scan is also possible for DMIs as we determined that aa changes at positions 313, 136 and 463 (or even combinations of these substitutions) are the most important substitutions causing reduced efficacy. In addition, promoter insertions can be visualized by a simple PCR and are important alerts for reduced efficacy of DMIs as recently

demonstrated for Costa Rica (Figure 8) (Diaz et al., 2016). Ideally, qPCR tests should be run directly on DNA preps from infected leaves to rapidly quantify and type DMI resistance in pathogen populations (Singh & Mustapha 2013). Eventually and evidently, sequence based technologies will revolutionize the discovery of underlying mechanisms of reduced sensitivities of disease control agents and will contribute to modern and optimized disease management.

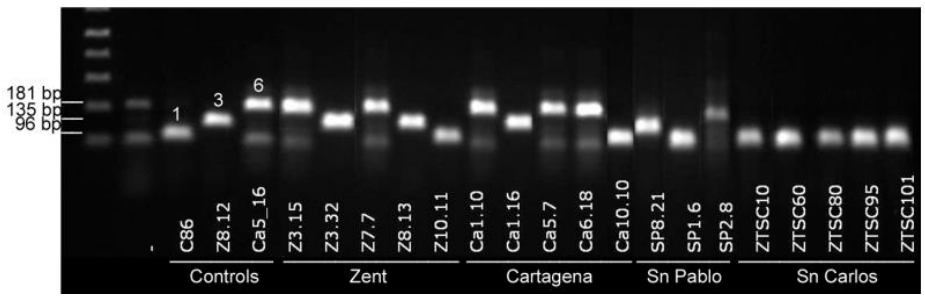

Figure 8. Example of PCR amplification of the *Pfcyp51* promoter in *Pseudocercospora fijiensis* isolates from different populations**.** Isolate CIRAD86 (C86) was used as indicative control for the presence of one promoter element, and isolates Z8.12 and CA5_16 as controls with three and six repeat elements, respectively. The number of repeat elements in each control sample is showed over the corresponding band. The other strains originate from banana plantations under fungicide disease management and represent various promoter length variants.

Comparing the use of DMI sensitivity data in the control of the fungal wheat pathogen *Zymoseptoria tritici* and *Pseudocercospora fijiensis*

P. fijiensis and *Z. tritici* are two of the most economically important pathogens of banana and wheat, respectively (Cook *et al.* 2013; Dean *et al.* 2012; Kema 2009). The analogy between these related dothideomycetes (Arango *et al.* 2016) is key to the use of *Z. tritici* as a model for the molecular studies in *P. fijiensis* (Kema 2009; Stergiopoulos *et al.* 2014). There are also striking similarities and dissimilarities with regard to the evolution of DMI resistance in both species, which will increase the understanding of the phenomenon and how to deal with

it under practical conditions. Both diseases are foliar blights caused by species with striking similarities in lifestyle: a heterothallic bipolar mating system with both asexual and sexual reproduction that enables these pathogens to complete several sexual cycles per year, resulting in genetically very diverse and versatile populations (Arzanlou *et al.* 2010). Yet, despite numerous speculations, recent data have shown that the basis on the plant pathogen interaction is a classic gene-for-gene model with avirulence effectors and host receptors (Aouini 2016; Arango *et al.* 2016; Stergiopoulos *et al.* 2010). For both diseases, DMIs are the cornerstone of disease management (Cools & Fraaije 2013). The evolution of DMI sensitivity in *Z. tritici* is a continuous threat to growers and the agrochemical industry and therefore represents the best studied system in agriculturally important plant pathogenic fungi (Cools & Fraaije 2013). Similar to *P. fijiensis*, shifts in DMI sensitivity in *Z. tritici* populations have been gradual by nature and are therefore commonly attributed to polygenic mechanisms, including (i) alteration in the *cyp51* sequence, (ii) overexpression of the *cyp51*gene and (iii) ATP-binding cassette transporters and major facilitators, resulting in fungicide efflux (Chong *et al.* 2016c; Cools & Fraaije 2013; Cools *et al.* 2013; Cowen 2008).

With regard to modulation of the *cyp51* gene, many - similar - mutations have been identified for *P. fijiensis* and *Z. tritici* (Cañas *et al.* 2009; Chong *et al.* 2016b; Cools & Fraaije 2013). In the *cyp51* gene for 36 amino aa substitutions were identified in *Z. tritici* and 28 aa in *P. fijiensis.* Some of these, identical, substitutions have been instrumental for DMI resistance (Chong *et al.* 2016b; Cools & Fraaije 2013). For example, the substitution Y136F in *P. fijiensis* is equivalent to Y137F in *Z. tritici*, and both are also linked with reduced DMI sensitivity in *Penicillium italicum, Uncinula necator* and *Blumeria graminis* f. sp. *hordei* (Albertini *et al.* 2003; Cools & Fraaije 2013; Délye *et al.* 1997). A substitution at Y136, or its equivalent in other species, is the most frequently observed modification of CYP51 in pathogenic fungi

(Cools *et al.* 2013). Interestingly, the equivalent position in *Z. tritici*, Y137F, although common in strain from the 1990s, has now virtually disappeared from the population. Cools and Fraaije (2013) associate this phenomenon with the disappearance of triadimenol, a fungicide that was commonly used in 1970s (Cools & Fraaije 2013). Substitution Y136F in *P. fijiensis* represents a 17.47% share in a recent global survey among 269 isolates and is primarily present in Costa Rica (2014) and Colombia (2012) (Chong *et al.* 2016b). It was also identified in two isolates from the Dominican Republic and in one strain from Cameroon (2014), but is absent in Ecuadoran isolates (2011) as well as strains from Martinique and Guadalupe (2013) (Chong *et al.* 2016b). Given the example of *Z. tritici*, it is of interest to study whether these differences can also be attributed to the use of particular fungicides in these countries. Substitutions V136A and I381V are correlated with reduced sensitivities to tebuconazole in *Z. tritici* (Cools *et al.* 2013). Positional changes at 380 and 381 in *P. fijiensis* tend to be rare, and were most prevalent in strains from Costa Rica and the Dominican Republic (Chong *et al.* 2016b). It would be of interest to test such isolates for sensitivity towards tebuconazole, but thereby considering that isolates that overexpress *Pfcyp51* are less susceptible and show reduced variation in their response (overexpression is not selected for based on specific DMI fungicides) (Cools & Fraaije 2013). Hence, any phenotypic test with tebuconazole should be conducted with a strain carrying substitutions at position 380 or 381, but with a wild type promoter.

Other similitudes are found in substitutions around positions 313 and 460 to 463 (Cools *et al.* 2013). In *Z. tritici* modulation of position 312 is rare, but aa changes at position 313 are ubiquitous in *P. fijiensis* (Chong *et al.* 2016b; Cools & Fraaije 2013). In both species we have identified numerous aa changes that do not have any apparent relation with DMI sensitivity and could either result from compensating mutation events, contingent evolution or exert pressure for the selection of important substitutions (Chong *et al.* 2016b; Cools & Fraaije

2013). CYP51 complementation experiments in *Saccharomyces cerevisiae* or *P. fijiensis* transformation experiments can be used to analyse the importance of these substitutions (Cools & Fraaije 2013; Díaz-Trujillo *et al.* 2016a). The collected data in both species clearly indicate that accumulation of mutations in the *cyp51* gene in drive reduced sensitivity to DMI fungicides (Cools *et al.* 2013). The environmental exposure of other fungi, including the human pathogen *A. fumigatus*, and the reduced efficacy of (medical) DMIs is a worrying situation with far reaching consequences for patients and potential risks for occupational health of workers in the agricultural sector (Risède *et al.* 2010; Snelders *et al.* 2012; Verweij *et al.* 2009).

Overexpression of the *cyp51* gene has been also reported in both species (Chong *et al.* 2010; Cools *et al.* 2012; Díaz-Trujillo *et al.* 2016a). In *Z. tritici* a 120 bp insertion in the promoter region correlates with a 10 to 40-fold overexpression of the *cyp51* gene (Cools *et al.* 2012). Similarly, promoter insertions in *Pfcyp51* cause an overexpression resulting in decreased sensitivity to DMIs (Díaz-Trujillo *et al.* 2016a). However, promoter insertions are very rare in *Z. tritici* but, contrastingly, common in *P. fijiensis* where the actual insertion is a repeat of a normal *Pfcyp51* promoter element of 19 bp that is repeated many times at position 103, upstream of the coding region of the *Pfcyp51* gene (Chong *et al.* 2016b; Díaz-Trujillo *et al.* 2016a). A similar tandem repeat associated reduction of DMI sensitiveness was observed in *A. fumigatus* (Mellado *et al.* 2007; Snelders *et al.* 2012; Verweij *et al.* 2013). The size, nature and location of the promoter inserts in *Z. tritici* and *P. fijiensis* are distinct and unique and therefore likely not related or due to a similar mechanism. Nevertheless, the fact that promoter insertions arose in three fungi that are commonly treated with DMIs raises important questions on their origin and role in order to improve their management both in agriculture as well as the clinical practice.

Finally, the role of transporters– either major facilitators (MFS) or ATP-binding cassette (ABC) transporters – in reduced efficacy due to increased efflux of active ingredients of fungicides has been reported in several plant and human pathogens, including *Candida albicans, A. fumigatus* and *Z. tritici* (Cools *et al.* 2013; Cowen 2008; Stergiopoulos *et al.* 2002; Zwiers 2002). Until now there is no report on increased expression of membrane transporters in *P. fijiensis*. All current evidence points to *cyp51* as the major regulator of DMI sensitivity (Chong *et al.* 2016c). Recently, Chong et al. (2016b) followed an unbiased genetic mapping approach to identify genomic regions involved in DMI sensitivity, which identified one major genetic window containing *Pfcyp51* on putative chromosome 7. However, the aforementioned genetic window contained at least 52 other genes, including a putative ABC transporter that cannot be ruled out to affect DMI sensitivity and await functional analysis (Chong *et al.* 2016c).

The *P. fijiensis* – banana interaction and its epidemiology impact on black Sigatoka disease

As pointed out above, recent studies have shown that the *P. fijiensis* homologue of the *Cladosporium fulvum Avr4* effector is recognized by the tomato *Cf4* resistance gene (Stergiopoulos et al., 2010; Arango et al., 2016). Moreover, the allelic variation at this *PfAvr4* locus is limited to just six variants (Stergiopoulos et al., 2014). Since Calcutta 4 (*M. acuminata* ssp. *burmannica*, a wild diploid banana) showed a typical hypersensitive response to *P. fijiensis* isolates carrying *PfAvr4*, it is considered that wild banana germplasm carries homologues of *Cf4* that can be used in either classical breeding or genetic engineering approaches (Arango et al., 2016). Similar to the situation in *Z. tritici*, a basic understanding of the pathosystem will eventually lead to enhanced breeding efforts that will lead to the discovery of new resistance genes for black Sigatoka management. Thus far, this is not seriously addressed and hence, all

breeding efforts rely on just natural infection. A more targeted approach taking into account the achievements in other similar pathosystems, such as the wheat-*Z. tritici* pathosystem (Mirzadi Gohari *et al.* 2015), will greatly advance breeding output and efficiency, which eventually will exploit natural host resistance as a major factor for disease control. Surprisingly, this is hitherto virtually neglected as a breeding target in banana.

The long road to a sound black Sigatoka disease management

First of all, it is important to note that black Sigatoka is primarily a problem in large monoculture export plantations that are dominated by "Cavendish" clones. However, the disease is definitely also of importance for non-export countries such as India and Brazil that either grow increasing volumes of "Cavendish" clones, or a suite of different varieties with greater appreciation by the consumer, respectively. Backyard farmers' crops may be affected by the disease as well, but need a completely different approach through targeted small-holder oriented programs. These are increasingly driven by commercial breeding companies, as for other tropical crops, including potatoes, cucurbits and peppers, see also http://www.accesstoseeds.org. In any case, host resistance is a cornerstone for appropriate, effective disease management and this philosophy is absent in the global banana industry, where black Sigatoka is considered as a disease one can deal with due to the fungicide solution, despite the enormous costs (on average at least 1,000 – 1,500 USD.ha^{-1}.year^{-1}). However, any political decision or consumer preference to reduce the chemical load in banana production or a legal abandonment of aerial spraying will directly affect the industry and call for more sustainable ways to control the disease. Instead of short-sightedness, the industry needs strategy and vision. This warrants increased efforts to professionalize breeding programs supported by sound

scientific data. The recent suite of papers on black Sigatoka disease management and biology contribute to this raising awareness (Chong *et al.* 2016b; Chong *et al.* 2016c; Díaz-Trujillo *et al.* 2016a).

The current review and the research referred to clearly indicate that the ceiling of chemical control of black Sigatoka is approaching or has been reached in some cases. One can simply not spray banana crops on a daily basis. Hence, alternative products with different moa´s and broader, multisite targets are indispensable for continued disease control. Meanwhile, sensitivity monitoring has to undergo a major shift towards scientifically oriented strategies using the latest technologies rather than old-fashioned worn-out methodologies that exist purely at the expense of production zones in the developing world. The continuously increasing resistance to systemic fungicides in the field is a clear wake-up call for the industry that now progressively falls back on protectants that are both environmentally unfriendly and threaten occupational health of farm workers, employees and surrounding villagers (van Wendel de Joode *et al.* 2016). Hence, governments, industry and the logistic chain players have to acknowledge and consider their responsibilities and undertake actions to ensure the trialling and release of new systemic fungicides and their integration with appropriate control strategies. In addition, the power of the retail sector and eventually of primarily Western consumers has to be leveraged with programs that constructively connect actors in banana production and the trade and simultaneously dovetails programs to support sound and sustainable banana production with justified wages for all chain participants. A low price in Western supermarkets at the expense of low wage countries is old fashioned, unjustified and conflicts with the current view on the distribution of wealth and harmonized good agricultural practice. However, strategic changes and solutions - such as diversified resistant banana germplasm - will only slowly surface, simply because these will take time and require substantial budgets to

materialize. Technically, major improvements of disease control methods can rapidly be achieved by (enabling the) adoption of new discoveries and using these in overall decision support systems as outlined above (Lapeyre, 2010; Risède et al., 2010). For instance, disease forecasting programs will work appropriately under suboptimal conditions for black Sigatoka disease development such as the dry tropics (Lapeyre, 2010). The challenge is to translate these to the wet tropics where *P. fijiensis* thrives and continuously undergoes sexual reproduction turning it into an extremely versatile pathogen. There, continuous monitoring of the fungicide sensitivity is one of the major tools for the timely and accurate modulation of forecasting and control strategies, which can be aided by the application of accurate and rapid molecular monitoring tools such as PCR-based technologies that will extend the timeframe for an adequate supervised response. Lessons from other crops should be learned in banana cropping thereby assuring a continued and justified access to food and fruit.

Supporting information

Table S1.- Example of fungicide application data sheet for the San Pablo plantation in Costa Rica.

DIRECCIÓN DE INVESTIGACIONES-CORBANA ASISTENCIA TÉCNICA EN EL MANEJO DE LA SIGATOKA NEGRA REGISTRO DE ASPERSIONES											
	Farm: San Pablo Este										Year: 2015
	Program		Operation		Oil	Oil	Cycle		$ (Irrigation)		
Week	Date	Fungicide Mix	Date	Mix	L/ha	Mz	Nu.	D.C.	ha cycle	/ha/pdo.	Observations
52/14					142.5	26.0	50				System. aplic.: Triaz.= 7, Aminas= 13, Benz.= 0
											Pyri.= 4, Carboxa= 1 y Estrob.= 2. Total= 13
1	28-Dec	Dithane 1,75+3+NF	Dec 28	D1,75+NF	3	0.92	1	6			MaxiBoost (0,6) / 161 ha
1	3-Jan	Dithane 1,75+4+NF	Jan 3	D1,75+NF	4	0.92	2	6			TechnoZn (1) / 157 ha
3	12-Jan	CSiCa2+9 (Calixin + Sico)	12	CSiCa2	9	1.00	3	9			155 ha
4	21-Jan	Volley (fenpropimorph) + Dithane 1+1,9+8	22	CVo(1)1,9	8	1.00	4	10			152 ha
5	27-Jan	Dithane 1,75+3+NF	27	D1,75+NF	3	0.92	5	5			TecnokZn (1) / 147 ha (renov c. 14-19)
5	31-Jan	Dithane 1,75+3+NF	31	D1,75+NF	2	0.92	6	4			Phytocrop (1) / 147 ha
7	9-Feb	Opus (epoxiconazole) Impulse (Spiroxamide) Dithane 1,9+9	Feb. 8	COpIm1,9	9	1.00	7	8			Adel x progr / 148 ha
8	18-Feb	Impulse(Spiroxamide) Siganex (AP) Dithane 1,9+9	18	CImSx1,9	9	1.00	8	10			147 ha
9	24-Feb	Dithane 1,75+3+NF	24	D1,75+NF	3	0.92	9	6			TecnokZn (1)
10	1-Mar	Dithane 1,75+3+NF	Mar. 3	D1,75+NF	4	0.92	10	7			Atra x clima / MaxiBoost (0,6)
11	10-Mar	CVo 0,85+1,9+6 (fenpropimorph + mancozeb)	10	CVo0,85+1,9	7	1.00	11	7			
11	14-Mar	Dithane 1,75+2+NF	14	D1,75+NF	2	0.92	12	4			TecnokZn (1)
12	21-Mar	CSx1,9+6 (pyrimethanil + mancozeb)	21	CSx1,9	6	1.00	13	7			
13	26-Mar	Dithane 1,75+3+NF	26	D1,75+NF	3	0.92	14	5			NutriProtector (0,51)
14	31-Mar	Dithane 1,75+3+NF	31	D1,75+NF	2	0.92	15	5			Psac (1)
15	9-Apr	CTiCa1,9+7 (mancozeb +tilt +tridemorph)	Apr. 9-12	CTiCa1,9	7.5	1.00	16	9			Ti= Tilt / Atra x clima / Día 12, aceite 8

DIRECCIÓN DE INVESTIGACIONES-CORBANA ASISTENCIA TÉCNICA EN EL MANEJO DE LA SIGATOKA NEGRA REGISTRO DE ASPERSIONES

Farm: **San Pablo Este** **Year: 2015**

	Program		Operation		Oil	Oil	Cycle		$ (Irrigation)		
Week	Date	Fungicide Mix	Date	Mix	L/ha	Mz	Nu.	D.C.	ha cycle	/ha/pdo.	Observations
16	15-Apr	Dithane 1,75+2+NF (Mancozeb)	18	CImSx1, 9	8	1.00	17	9			Reprog x atra y clima
17	23-Apr	Dithane 1,75+2+NF (Mancozeb)	23	D1,75+NF	2	0.92	18	5			Día 25 renov 2014: Sx+5 (19,5 ha) / FolivFe (1)
18	28-Apr	DBg1,5+1 (Mancozeb)	28	DBg1,5+1	0	0.79	19	5			Psac (1)=$4,80
19	3-May	D43Br1,35+0,5 (Dithane + Bravo)	May 3	D43Br	0	0.10	20	5			1,35+0,5 / TecnoZn (1)
20	8-May	Br1,2 (Chlorothalonil)	10	D1,75+NF	2	0.92	21	7			Atra y reprog x clima
21	20-May	Regnum Calixin Dithane 1,9 + 9	21	CRgCa1,9	9	1.00	22	11			Atra x clima
22	30-May	Impulse Siganex Dithane 1,9+8	30	CImSx1, 9	8	1.00	23	9			
23	4-Jun	Dithane 1,75+2+NF	Jun. 4	D1,75+NF	2	0.92	24	5			Psac (1)
24	9-Jun	Dithane 1,75+2+NF	9	D1,75+NF	2	0.92	25	5			
25	14-Jun	Dithane 1,75+2+NF	14	Po1,35+NF	2	0.00	26	5			x falta de Dith 60 /
27	20-Jun	Dithane 1,75+2+NF	28	CSvIm1, 9	9	1.00	27	14			Banazeb/Reprog x clima/Rep espec 16-17 S (Sx0,25, 19 ha)
29	9-Jul	Cumora Siganex Banazeb 2+9	Jul. 14	CCuSx2	9	1.00	28	16		1 - 5	Atra x clima
29	13-Jul	Bb1,75+2+NF (Mancozeb)	18	Bb1,75+NF	2	0.92	29	4			Bb=Banazeb (mancozeb)/ MaxiB+Psac (0,5+0,56)
30	23-Jul	BbNTZn1,5+1,5+4+NF (Mancozeb)	24	BbNZn+NF	4	0.92	30	6	92		NZnP (cera) (1,5) / Atra x clima
31	1-Aug	Volley (1) Banaz 2+9	Agu. 1	CVo1+1, 9	9	1.00	31	8			
33	10-Aug	Sico Calixin Banaz 1,9+9	11	CSiCa1, 9	9	1.00	32	10		5 - 12	
33	16-Aug	BbNZn1,5+2+3+NF	17	BbNZn+NF	4	0.86	33	6			Atra x clima/NZn:1 v x proveedor/BbNF1,75+0, 3:1 v
34	22-Aug	Dithane 1,75+3+NF	22	Bb1,75+NF	3	0.92	34	5			Psac (1)
35	26-Aug	Dithane 1,75+3+NF	26	Bb1,75+NF	2	0.92	35	4			TechnoCa (1)

DIRECCIÓN DE INVESTIGACIONES-CORBANA ASISTENCIA TÉCNICA EN EL MANEJO DE LA SIGATOKA NEGRA REGISTRO DE ASPERSIONES

Farm: **San Pablo Este** **Year: 2015**

	Program		Operation		Oil	Oil	Cycle		$ (Irrigation)		
Week	Date	Fungicide Mix	Date	Mix	L/ha	Mz	Nu.	D.C.	ha cycle	/ha/pdo.	Observations
36	3-Sep	Impulse Siganex Banaz 1,9+8	Set. 3	CImSx1, 9	8	1.00	36	8			
37	8-Sep	BbNZn1,5+2+3+NF	10	BbNZn+ NF	5	0.79	37	7			NTZnP (2) / Sin Mist Control
38	17-Sep	Volley Banaz 1,9+7	17	CVo1,9	7	1.00	38	7		14	
39	22-Sep	Bb1,75+3+NF	22	BbPhCu	3	0.26	39	5			0,50 + PhCu 0,53
39	27-Sep	Bb1,75+3+NF	27	Bb1,75+ NF	3	0.79	40	5			Tecamin (1)
41	5-Oct	Tilt Calixin Banaz 1,9 +8	Oct. 6	CTiCa1, 9	8	0.92	41	9		6 - 15	
41	10-Oct	BbNZn1,5+2+3+NF	10	BbNZn+ NF	2	0.79	42	4			
42	15-Oct	Bb1,75+3+NF	15	Bb1,75+ NF	3	0.92	43	5			FolCa+Everest
43	23-Oct	Volley Banaz 1,9+7	23	CVo1,9	7	1.00	44	8			Naturamin (150 g)
44	29-Oct	BbNZn1,5+2+3+NF	29	BbNZn+ NF	2	0.79	45	6			
45	4-Nov	Bb2+2 (Macozeb)	Nov. 4-5	Bb2	2	0.92	46	6			Prueba con fruta sin bolsa y agua de reuso
46	10-Nov	Bb1,75+3+NF	10-12	Bb1,75+ NF	2	0.92	47	6			TechnoCa+ NitK (1+1) / Atra x clima / 12: aceite 3
48	16-Nov	Bb1,75+3+NF	17	Bb1,75+ NF	4	0.92	48	6			
48	25-Nov	Regnum Siganex Banaz 1,9 + 8	26	CRgSx1, 9	8	1.00	49	9			Atra x clima
49	5-Dec	Opus Impulse Banaz 1,9+8	Dec. 4	COpIm1, 9	8	1.00	50	9			
50	11-Dec	Bb1,75+2+NF	10	Bb1,75+ NF	2	0.92	51	6			
51	15-Dec	BbNZn1,5+1,5+2+MC	15	BbNZn+ MC	2	0.79	52	5			
52	20-Dec	Bb1,75+2+NF (0,20)	23-24	CImSx1, 9	8	1.00	53	8			Reprog x clima y atraso
CCa=Calixin + Dithane, Rg= Regnum, CSx=Siganex + Dithane, D=Dithane, Br=Bravo											Sistém. aplic.:Triaz.= 7, Aminas= 18, Benz.= 0
				Subtotal:	251.5	47.1	53				Pyri.= 6, Carboxa= 1 y Estrob.= 2. Total= 21
				Total:	298.6						

Chapter 3

Global analysis of reduced sensitivity to azole fungicides in the banana black Sigatoka pathogen *Pseudocercospora fijiensis*

Pablo Chong[1,11], Josué Ngando Essoh[2,10], Rafael Arango[3], L. C. Paul Keizer[4], Ioannis Stergiopoulos[5], Michael F. Seidl[6], Mauricio Guzman[7], Jorge Sandoval[7], Paul E. Verweij[8], Gabriel Scalliet[9], Helge Sierotzski[9], Luc de Lapeyre de Bellaire[10], Pedro W. Crous[6,12], Jean Carlier[11], Sandrine Cros[10], Harold J. G. Meijer[13], Esther Lilia Peralta[1] and Gert H. J. Kema[6,13]

Abstract

Pseudocercospora fijiensis is the causal agent of black Sigatoka or black leaf streak disease of bananas and plantains. Due to the overall susceptibility of the main export Cavendish bananas, black Sigatoka management largely relies on fungicides, predominantly on multisite inhibitors and azoles, which belong to the sterol demethylation-inhibitors (DMIs) that target the lanosterol 14α-demethylase enzyme CYP51. We examined the azole sensitivity of 592 field isolates of *P. fijiensis* collected from various banana production zones in Colombia, Costa Rica, the Dominican Republic, Ecuador, the Philippines, Guadalupe, Martinique and Cameroon. Continuous sensitivity ranges towards the DMIs fungicides difenoconazole, epoxiconazole and propiconazole was observed with clear patterns of cross-sensitivity. Genotyping by sequencing was applied to study the overall genetic diversity in a geographical subset of 155 *P. fijiensis* strains, which revealed a distinct clustering based on the geographical origins of the isolates, with clear subclades for African, Latin American and the Caribbean isolates. Finally, sequence analyses of the CYP51 encoding gene *Pfcyp51* in 266 isolates showed a wide suite of mutations. Twenty-eight independent point mutations result in amino acid (aa) substitutions with nine of them correlating with reduced sensitivity to DMIs. Moreover, we identified nine novels regionally disseminated aa substitutions. The majority of the substitutions correlated with reduced sensitivity to DMIs are in the proximity or affect the putative substrate-binding site based on *in silico* predictions of the CYP51 protein models. In addition, up to six – sometimes unique - insertions in the *Pfcyp51* promoter could be found in strains displaying reduced azole sensitivity. Such promoter insertions correlate with reduced DMI sensitivity and, frequently contain repeated elements with a palindromic core. Wild type strains from unsprayed bananas in Ecuador, Colombia and Cameroon did not contain any promoter insertions. Our study is the first global analysis of fungicide resistance in *P. fijiensis*,

and provides a lead to understand DMI sensitivity reduction, and enables the development of better black Sigatoka management strategies, but also calls for the deployment of a wider range of solutions for a sustainable control of this unparalleled banana threat.

Introduction

Banana is an important staple food (plantain AAB, 2n=3x=33; cooking banana ABB, 2n=3x=33)(D'Hont *et al.* 2012; Perrier *et al.* 2011) and the most popular fruit (dessert banana usually AA or AAA, 2n=2x=22 and 3n=3x=33, respectively)(Ploetz *et al.* 2015) around the world. Commercial banana production is dominated by "Cavendish" cultivars that almost exclusively comprise the export trade (95%), but that are also increasingly important for domestic markets in many countries, such as India and China (Ploetz *et al.* 2015). Moreover, Cavendish plantations are actively developed in the Middle-East and East Africa as an important cash crop (Shively & Hao 2012; Zeitoun *et al.* 2012). The success of Cavendish clones is largely explained by their resistance to Panama disease that wiped out "Gros Michel" banana cultivar in Central America in the previous century. However, banana production using "Cavendish" clones also facilitates the dissemination of a new Panama disease causing strain of the soil-borne *Fusarium oxysporum* f.sp. *cubense* (the so-called Tropical Race 4 strain, (Ordoñez *et al.* 2015)) that threatens global banana production. A major foliar blight affecting global banana and plantain production is black Sigatoka or black leaf streak disease, which is caused by the dothideomycete fungus *Pseudocercospora fijiensis* (previously *Mycosphaerella fijiensis*). Contrary to Panama disease, *P. fijiensis* colonizes and destroys the foliage by developing characteristic necrotic spots that eventually coalesce in large blotches that destroy the leaves (Figure S1), thereby initiating physiological adaptations that results in premature

fruit ripening, which is a major secondary post-harvest loss (Stover & Simmonds 1987). Due to the extreme susceptibility of "Cavendish" bananas, black Sigatoka is considered as the costliest banana disease requiring extraordinary fungicide input that threatens the environment and affects the occupational health of plantation workers (Risède *et al.* 2010; van Wendel de Joode *et al.* 2016). The increasing fungicide applications (Chong *et al.* 2016a; de Lapeyre de Bellaire 2009) exert an enormous selection pressure on *P. fijiensis* populations that gradually affect the efficacy of the applied fungicides. Sterol demethylation-inhibitors (DMIs) are the commonest applied systemic fungicides for black Sigatoka management (Cañas *et al.* 2009). These fungicides interfere with the catalytic site of the lanosterol 14α-demethylase enzyme, also known as CYP51 (Cañas *et al.* 2009), which is a key player in ergosterol biosynthesis by catalysing the demethylation of lanosterol via its heme bound iron atom in the substrate recognition site (SRS) (Akins & Sobel 2009; Lepesheva & Waterman 2004; Warrilow *et al.* 2013). The continuous and massive use of DMI fungicides has contributed to the selection of reduced sensitivity and eventual resistance in *P. fijiensis* populations (Cañas *et al.* 2009; Chong *et al.* 2016a; Churchill 2011a; Guzmán *et al.* 2013; Marín *et al.* 2003; Ploetz 2000). Selection and concurrent spread into and across *P. fijiensis* populations highly depends on the applied fungicides and the properties of the pathogen population (Lapeyre *et al.* 2010b; Robert *et al.* 2012; Vincellin 2014). The link between DMI fungicides overuse and the occurrence of reduced efficacy and concurring genetic variation at the target site has been demonstrated in many fungal species (Becher & Wirsel 2012; Cools *et al.* 2013; Villani *et al.* 2016; Warrilow *et al.* 2013). The commonest observed genetic mechanisms of DMI resistance are non-synonymous point mutations in the coding region of the *cyp51* gene resulting in modified versions of the CYP51 protein, and changes in the *cyp51* gene promoter resulting in elevated expression levels (Akins & Sobel 2009; Albarrag *et al.* 2011; Albertini *et al.* 2003; Bean *et al.* 2009; Bolton *et al.* 2016; Cools *et al.* 2012; Cools *et al.* 2013; Délye *et al.* 1997; Díaz-Trujillo

et al. 2016a; Dyer *et al.* 2000; Eddouzi *et al.* 2013; Hamamoto *et al.* 2000; Ma *et al.* 2006; Mellado *et al.* 2007; Schnabel & Jones 2000; Verweij *et al.* 2013). Point mutations in the *cyp51* coding region mostly result in amino acid (aa) changes within the six SRS (SRS1-6) regions (Cañas *et al.* 2009; Lepesheva & Waterman 2004), which are peptide chains regions at the protein core that interact with the target substrate. The mentioned substitutions do not inactivate the enzyme but compromise fungicide binding affinity (Cools *et al.* 2012; Lepesheva & Waterman 2004). The most common substitutions in the *P. fijiensis cyp51* gene (*Pfcyp51*) are at positions Y136 and A313, inside the putative SRS1 and SRS4 respectively, and substitutions Y461 and Y463 (Cañas *et al.* 2009; Chong *et al.* 2010). Interestingly, *P. fijiensis* isolates from Costa Rica with an accumulated number of mutations in the *Pfcyp51* gene also contain promoter insertions (Díaz-Trujillo *et al.* 2016a). The insertions in the *Pfcyp51* promoter are composed of repeated elements. Promoter replacement analysis showed that these repeats alone are responsible for increased EC_{50} values (Díaz-Trujillo *et al.* 2016a).

Although there is information regarding the genetic variation of *P. fijiensis* at specific geographical locations (Halkett *et al.* 2010; Hayden *et al.* 2003; Robert *et al.* 2012), the relationship between genetic diversity with DMI usage is currently lacking. Here, we analyse the molecular effects underlying reduced sensitivity and resistance towards DMI fungicides by phenotyping the azole sensitivity of 592 isolates. These data are further supported by sequencing the *Pfcyp51* gene and promoter region of a 266 isolate subset, collected worldwide from major banana producing countries. Furthermore, we show a positive correlation between increased DMI applications, the presence of specific genetic modifications in the promoter and coding region mutations of *Pfcyp51* and reduced azole sensitivity. We also modelled the impact of amino acid changes at the substrate recognition site of the PfCYP51 protein, indicating which mutations possibly contribute significantly to azole resistance. Our findings

support the hypothesis that DMIs exert a stringent selective pressure on *P. fijiensis* in banana plantations globally.

Materials and methods

Pseudocercospora fijiensis strains and inoculum

A suite of 592 *P. fijiensis* strains from major banana producing and indigenous regions in Africa, Asia and Latin America was collected and analysed in this study (Table 1). A random set of strains from this global collection was tested for confirm their specie identity based on the elongation factor-1α sequence, which was amplified with primers EF1-728F (5′-CATCGAGAA GTTCGAGAAGG-3') and EF1-986R (5′-TACTTGAAGGAACCCTTACC-3') (Carbone & Kohn 1999) and analysed using the NCBI genome database and the *P.(Mycosphaerella) fijiensis* v2.0, JGI genome portal.

Originally, 612 isolate were collected but we were unable to recover 20 *P. fijiensis* isolates from the collection preserved (Preserving solution: 50% of potato dextrose broth and 30% glycerol) at -80°C, and hence 592 *P. fijiensis* isolates were available for subsequent phenotyping. From this set 266 isolates were selected based in their DNA quality and their phenotyping for genotypic analyses, including strains from which we had genomic DNA (gDNA) or *Pfcyp51* sequences available. Five sensitive isolates (X845, X846, X847, X849 and X851) were used to compare the sequence variation among *Pfcyp51* wild type genes but were not phenotype in this study. We regarded these strains as DMIs sensitive, based on available information for their response to propiconazole (Díaz-Trujillo *et al.* 2016a).

Table 1. Origins and characteristics of the *Pseudocercospora fijiensis* isolates used in this study.

Country / collection	Year of collection	Isolates DMI sensitivity tested	*Pfcyp51* Sequenced	Population characteristics	DMI and total fungicide application per year of collection
Colombia CIB UBALMED	late 2012	98	34	Treated farms and a subset of 13 isolates from non-treated zones	DIM estimated application: 7 cycles from a total of 32 cycles
Costa Rica CORBANA	early 2014	107	33	Treated farms	DIM estimated application: 7 cycles from a total of 56 cycles
Dominican Republic CIRAD	early 2013	25	23	Treated farms	Data undetermined
Ecuador CIBE-ESPOL	early 2011	101	40	Treated farms and a subset of 25 isolates from non-treated zones	DIM estimated application: 13 cycles from a total of 30 cycles
Philippines PRI-WUR	early 2013	98	28	Treated farms	DIM estimated application: 12 cycles from a total of 54 cycles
Guadalupe CIRAD	early 2013	30	3	Non-treated (low exposure)	DIM estimated application: 6 cycles from a total of 10 cycles
Martinique CIRAD	early 2013	42	5	Non-treated (low exposure)	DIM estimated application: 9 cycles from a total of 11 cycles
Cameroon CIRAD	midst 2014	90	94	Treated farms and a subset of 25 isolates from non-treated zones	DIM estimated application: 7 cycles from a total of 45 cycles
Individual sensitive isolates* WUR	2009	1	6	Non-treated	Non-treated zones
Total:	8 collections	592	266		

**(Indonesia, Gabon, Burundi, Taiwan, Philippines and Cameroon)*

Inoculum preparation

Inocula were prepared by using the protocol of Peláez et al. (Peláez *et al.* 2006) with modifications. In short, a piece of mycelium (~0.5 cm^2) from a 3-4 weeks old *P. fijiensis* colony grown on potato dextrose agar (PDA) medium was blended for 20 sec. at 6,000 rpm in an Ultra Turrax Tube Drive homogenizer (IKA, Staufen, Germany) using a sterile DT-20 tube (IKA, Staufen, Germany) in 15 ml of distilled water. Mycelial pieces were filtered through the Steriflip Vacuum-driven Filter System (Sterile 100 µm; Merck Millipore, Billerica, USA) and quantified in a Kova glasstic slide 10 with a grids coverslip microscope slide (Kova,

California, USA). The mycelial fragment concentration was adjusted to approximately $5x10^5.ml^{-1}$.

Fungicide testing

Syngenta Crop Protection AG, Basel, Switzerland, provided technical grade quality fungicide samples of propiconazole and difenoconazole. Epoxiconazole was obtained from Sigma (Sigma Aldrich, Missouri, USA). The propiconazole and difenoconazol azoles were maintained as 50.000x stock solutions and epoxiconazole as a 20.000x stock solution in DMSO. Fifty µl of mycelium solution was mixed with 200 µl PDB medium supplemented with antifungal compounds in flat bottom transparent polystyrene non-coated 96-wells microtiter plates (Corning, New York, USA).

Each strain was initially tested in duplicate, against seven concentrations (0.004, 0.016, 0.04, 0.16, 0.64, 2.56 and 10.24 $mg.L^{-1}$) for each fungicide and a water control. In a secondary screening, a selected subset - based on their geographical origin and sensitivity response - of 212 isolates was re-evaluated in at least three biological repetitions. Finally, a third test was performed for 21 DMI resistant *P. fijiensis* isolates (>10 $mg.L^{-1}$ in the initial test) against extended final concentrations using 0, 0.64, 2.56, 10.24, 15.36, 20.48, 30.72, 40.96 $mg.L^{-1}$. In all experiments, the final concentration of DMSO was kept at 1% (v/v) and plates were incubated in the dark at 27°C for 10 days. Mycelium growth was determined after removing the cover of the plates using a micro plate reader Infinite® 200 PRO machine, TECAN, Switzerland, which was calibrated at room temperature (wavelength 690 nm, multiple reads per well in a 5x5 circle-filled form, bandwidth 9 µm, number of flashes 5 and 1 mm exclusion from well walls). The concentration that resulted in 50% growth inhibition

(EC_{50}) was determined by plotting the growth profiles from the OD readings, adjusted for the background. Monotone regression spline functions (Ramsay 1988) were applied to fit the curve profiles using GenStat 18th Edition software (VSN International, Hemel Hempstead, UK). The EC_{50} sensitivity threshold ranges for all fungicides were arbitrary chosen based on the clustering analyses of the $^{2}\log EC_{50}$ means standard error of the differences and the genetic information of the *Pfcyp51* gene. The EC_{50} sensitivity thresholds selected for the strains grouping were: resistant >1 $mg.L^{-1}$, tolerant from 0.1 to 0.99 $mg.L^{-1}$ and sensitive <0.1 $mg.L^{-1}$.

Pfcyp51 sequencing

The coding region of the *Pfcyp51* gene and its promoter were amplified using the specific primers *CYP51_Pfijien_F1* (5'-AAGGTCATATCGCAGG-3') and *CYP51_Pfijien_R1* (5'-GAATGTTATCGTGTGACA-3'). The PCR program consisted of an initial denaturation step at 94°C for 5 min, followed by 34 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds and an extension at 68°C for 90 sec. A final extension step was performed at 72°C for 7 min. The expected amplicons ranged from 2 to 2,2 Kb and were directly sequenced by Macrogen (Seoul, Korea) using the amplification primers and additional sequencing primers: *CYP51_Pfijien_F2* (5'-ACAGAAACATCACCTCC-3', *CYP51_Pfijien_F3* (5'-ATTGCTTCACTTTCATCC-3'), *CYP51_Pfijien_F4* (5'-CTCTACCAC GATCTCGAC-3') and *CYP51_Pfijien_R2* (5'-GATATGGATATAGTTGT-3'). For each strain the sequences were assembled using SeqMan (Lasergene v8 software from DNASTAR®). Contigs were aligned and analysed using CLC Genomic software version 7.5.2 from Qiagen (Hilden, Germany). The wild type *P. fijiensis* strain CIRAD86 was used as reference to determine the number and type of mutations in each isolate. We used MEME

(Bailey & Elkan 1994), GLAM2 (Frith *et al.* 2008) and ESEfinder 3.0 (Cartegni *et al.* 2003) software to analyse the promoter region of *Pfcyp51*.

Model building and docking studies

The three-dimensional structures of seven PfCYP51 proteins (hybrid models) were predicted using YASARA software (http://www.yasara.org). The hybrid models, were predicted using a three-dimensional template of the CYP51 proteins from *Aspergillus fumigatus* (PDB code: 4UYM) (Hargrove *et al.* 2015), *Saccharomyces cerevisiae* (PDB code: 4LXJ and 4K0F) (Monk *et al.* 2013), *Homo sapiens* (PDB code: 3JUS) (Strushkevich *et al.* To be publish) and *Mycobacterium tuberculosis* (PDB code: 2W0B) (Chen *et al.* 2009). From each template five variant models were generated. Each variant model was scored with the Z-scores calculated from molecular dynamics force field energies. The variants with the best Z-scores were selected to build the final hybrid models. The crystal structure of the lanosterol 14α-demethylase (CYP51b) from *A. fumigatus* in complex with voriconazole was used as main template. The same software package was applied for simulating the docking of propiconazole in the SRSs of CYP51. The chemical structure of the tested fungicide propiconazole (PubChem code 43234), was retrieved from PubChem (http://pubchem.ncbi.nlm.nih.gov/compound/propiconazole). The global distance test was performed using default settings. Active side residues were defined as those within 7Å (Chen *et al.* 2010) of the substrate closest atom. The selected modelled genotypes are listed in Table S3.

DArTseq markers generation

A set of 155 *P. fijiensis* isolates were selected based on origin and DNA quality and genotyped using DArTseq sequencing technology (www.diversityarrays.com/). DNA samples were processed in digestion/ligation reactions as described before (Kilian *et al.* 2012). The technology was optimized for *P. fijiensis* by replacing a single *Pst*I-compatible adaptor with two separate adaptors corresponding to two different Restriction Enzyme (RE) overhangs. The *Pst*I-compatible adapter was designed to include the Illumina flow cell attachment sequence, a sequencing primer sequence and a "staggered" varying length barcode region (Elshire *et al.* 2011). The reverse adapter contained the flow cell attachment region and a *Mse*I-compatible overhang sequence so that only "mixed fragments" (*Pst*I-*Mse*I) amplify effectively by PCR.

Equimolar amounts of amplification products from each sample of the 96-well microtiter plate were bulked and applied to c-Bot (Illumina) bridge PCR followed by sequencing on an Illumina Hiseq2000 apparatus. Sequences generated from each lane were processed using proprietary DArT analytical pipelines (Kilian *et al.* 2012). In the primary pipeline, the fastq files were first processed to filter for poor quality sequences, applying more stringent selection criteria to the barcode region compared to the rest of the sequence resulting in reliable assignments of the sequences to specific samples. Approximately 2,000,000 sequences per barcode/sample were identified and used in marker calling. Identical sequences were collapsed into "fastqcoll files" and subjected to a second pipeline for further quality selection criteria (Kilian *et al.* 2012). Finally, the score markers (presence/absence of restriction fragments) were represented in a 0/1 binary matrix for usage in the genetic similarity calculation.

Population clustering analyses

To determine the genetic diversity of *P. fijiensis*, we utilized the DArTseq markers of the 155 isolates that originated from eight distinct geographical locations. DArTseq markers were quality filtered (Qpmr >2.7, Reproducibility =1, CallRate >0.66), resulting in 6,586 polymorphic DArTseq markers. Based on the presence or absence profiles of these markers, the Jaccard-distance between isolates was determined using R (http://www.R-project.org) (R-Development-Core-Team 2008). Subsequently, complete hierarchical clustering analysis was performed, as implemented in R (R-Development-Core-Team 2008).

Analyses of *P. fijiensis* strains with the sensitivity trait

For practical reasons, not all 592 isolates could be tested on three fungicides in replication, as is described above. A single estimate on all fungicides was made for 294 isolates, while for 253 isolates the EC_{50} was estimated in triplicate (for the majority). Only 45 isolates did not give a proper EC_{50} estimate to all fungicides. The data was first analysed with a full factorial ANOVA model comparing main effects and interactions for experimental factors isolates and fungicides. Prior to analysis the data were ^{2}log-transformed to obtain homogeneity of variance and a better approximation by the normal distribution. The interaction space of this ANOVA with (3-1).(592-1) parameters, if significant, can be described with more succinct models.

The Finlay-Wilkinson model (FW) (Eberhart & Russell 1966; Finlay & Wilkinson 1963) describes the interaction between two factors in a more parsimonious nonlinear form. It models one of the factors as a product with a linear relation to the other. This relation can depend either on the fungicide or isolate with EC_{50}; $y_{ijk} = Fungicide_i + b_i \times Isolate_j + \varepsilon_{ijk}$ or y_{ijk}

= $Isolate_i + b_i$ x $Fungicide_j + \varepsilon_{ijk}$. This results in 'sensitivities' (b_i) for fungicides or isolates indicated by the steepness of the slope. For isolates this results in nearly 600 lines as sensitivities of each isolate independently, while for fungicides it uses only three lines to describe the general sensitivity response towards each fungicide. Sensitivity above 1 means more sensitive and vice versa.

Analyses of the sensitivity trait with *Pfcyp51* mutations

From a subset of 266 isolates, 23 substitutions, binary variables, were established and a promoter palindromic factor with 6 levels (*Pfcyp51* sequencing). Included are the fungicide treatment and country as explanatory factors, with 3 and 8 levels. The FW estimates of the EC_{50} sensitivities were taken as the response or dependent variable in a regression model, with the mutations and promoter, country and fungicide are explanatory. To analyse main effects of the substitutions alone, these were first fitted with a step-forward approach to select the most explanatory ones without the expected moderating and/or confounded effect of the promoter or the other factors. These selected substitutions were subsequently subjected to an all-subset selection procedure, where we can decide which subset of significant substitutions forms the most stable combination. These most explanatory substitutions variables were used to refit the model, now with the promoter and fungicide factor added as main effects. In the next three steps, possible first order interaction terms with the mutations were added with forward selection followed by backward elimination. Each of these rounds tries iteratively to include subsequent interaction terms based on a forward inclusion ratio and overall significance and retains only the best fitting combinations. First among the mutations themselves, then mutations with promoter and finally mutations with fungicide and country. The model resulting from this process is refitted to arrive at a final model with backward

elimination to see if any previously included interaction terms have become superfluous. The 23 mutations were pairwise tested for interaction with Fischer's Exact test on independence, which can be used to judge the plausibility to accept or discard certain results from the subsequent model fitting.

Results

Pseudocercospora fijiensis specie confirmation

Different species of the fungal genus *Pseudocercospora* cause very similar symptoms on banana. Moreover, these species also morphologically resemble *P. fijiensis* and can coexist in the same leaf (Arzanlou *et al.* 2008; Churchill 2011a), the so-called black Sigatoka complex. We assessed the potential occurrence of other *Pseudocercospora* species in our global collection of isolates. We selected 28 strains from the collection on the basis of their colony morphology to sequence the elongation factor-1α gene to confirm their identification. PCR amplification resulted in fragments for all strains and, based on blast analyses we identified these strains as *P. fijiensis*, suggesting that most of the strains in the global collection were correctly identified based on morphology and ascospore germination patterns (data not shown).

Fungicide sensitivity of the *P. fijiensis* collection to DMIs

The *Pseudocercospora fijiensis* collection was tested for sensitivity against three DMI fungicides; difenoconazole, epoxiconazole and propiconazole (Table S1). In general, we observed cross-resistance among all strains for these three compounds. In Figure S2a the raw

^{2}log (EC_{50}) versus fitted estimates illustrates this as a positive band. Fitting the full factorial model revealed that there was a modest interaction between isolate and fungicide (p=0.027) at the cost of a huge number of parameters. A simpler model is the FW model, which describes the interaction variation together with one of the main effects as a linear product. If this factor was the isolate, the angle of the relation, expressed the sensitivity for of each isolate towards the fungicide compared to 1. These sensitivities were not significant (p=0.24), instead used for the fungicides it expressed the sensitivity of each fungicide toward all isolates and had much more explanatory power (p<0.001). Figure S2b shows this FW model with 3 lines, based on the isolate means, shows clearly the interaction by the difenoconazole sensitivity line crossing the other two fungicides sensitivities which are nearly parallel, so behave additive. For that reason, the structure of the populations bases on their sensitivity response (resistant, tolerant or sensitive) might differ between products (Figure S2b and S3). In countries where banana production requires black Sigatoka management through frequent fungicide applications, viz. Costa Rica, Colombia and the Philippines, isolates with reduced sensitivity were clearly dominant, in decreasing and distinct order (Figure 1, Tables 2 and S1, S2). In countries such as Cameroon, the Dominican Republic and Ecuador where the use of DMIs is still relatively limited a majority of tolerant *P. fijiensis* isolates, was found (Tables 2 and S2). In contrast, all *P. fijiensis* isolates from Guadalupe and Martinique were sensitive (Tables 2 and S2). DMIs are used for disease control in both islands but since *P. fijiensis* recently arrived, the time of the exposure of the population to the DMIs have been short. The DMI sensitivity levels among *P. fijiensis* isolates found in Costa Rica are the lowest across all isolates, with no isolates classified in the sensitive category and isolates classified in the tolerant category ranging from one percent for propiconazole, two percent for difenoconazole and three percent for epoxiconazole with the rest of the isolates been resistant (Table S2).

Table 2. Fisher's protected least significant difference test showing the difference in sensitivity from *Pseudocercospora fijiensis* populations by origin.

Country	mean ^{2}log (EC_{50})	hom. group	Isolate count
Guadalupe	-6.015	a	30
Martinique	-5.833	a	42
Ecuador	-2.655	b	101
Cameroon	-2.655	b	90
Dominican R.	-0.924	c	25
Colombia	0.220	d	95
Philippines	0.388	e	98
Costa Rica	2.010	f	111

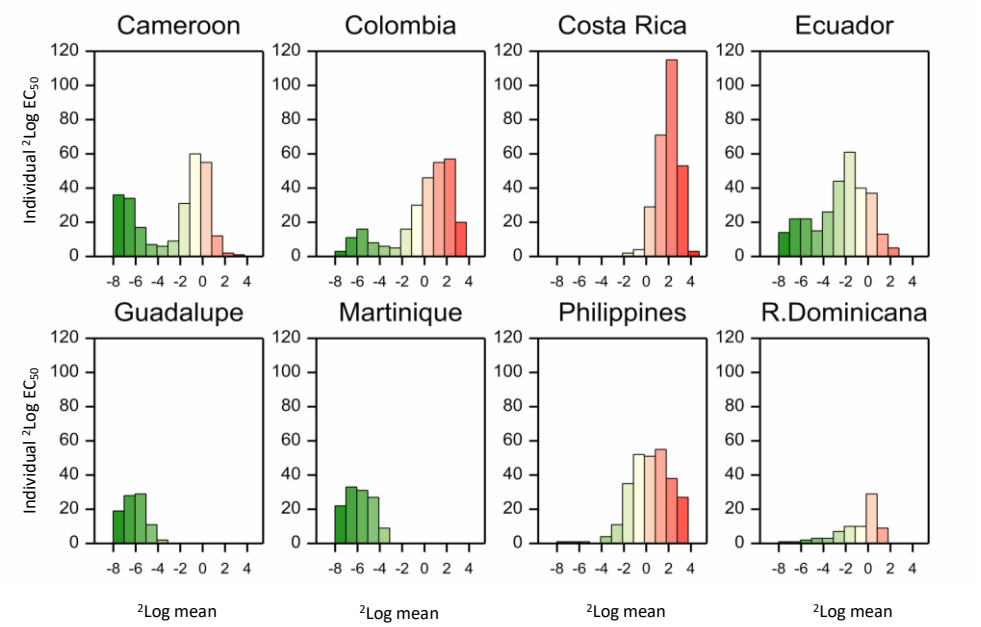

Figure 1. Observed sensitivity differences to three DMI fungicide (difenoconazole, epoxiconazole and propiconazole) among *Pseudocercospora fijiensis* strains from varying countries. Data are presented as the frequency of individual EC_{50} data that match against the EC_{50} means for the combined response to the tested DMIs (^{2}Log).

The EC_{50} values for Costa Rican *P. fijiensis* isolates for the three DMIs were the highest. The majority of isolates in Philippines and Colombia were also resistant. Isolates from Ecuador, Dominican Republic and Cameroon were mostly tolerant. Nonetheless, sensitive and resistant strains were also represented (Figure 1 and Table S2). The lowest values were found in isolates originating from Guadalupe, Martinique and Cameroon. All isolates from untreated areas from Cameroon, Colombia and Ecuador were also sensitive. (Figure 1 and Table S2). Interestingly, Costa Rica population (one of the main banana exporting countries) and the populations from Guadalupe and Martinique (with low fungicide exposure) perfectly fit with the chosen thresholds for DMI resistance and sensitive, respectively (Figure 1 and Tables 2 and S2). Other countries as Cameroon, Colombia and Ecuador have an almost continue set of values (Figure 1 and Table S2). The overall response of the global population is shown in Figure S2 and S3. The additional sensitivity analyses on 21 resistant strains with high fungicide dose (up to 40.96 $mg.L^{-1}$, Figure S4) revealed that CaM10_6, CaM1_5 and CaM3_1 from Costa Rica had extremely high EC_{50} values, especially in their response to difenoconazole and propiconazole (Figure S4).

DArTseq genotyping

We analysed the genetic variation among 155 isolates of *P. fijiensis* (Figure 2) using hierarchical clustering based on 6,586 polymorphic DArTseq markers. We detected a clear clustering pattern reflecting the geographical origin of the samples. For example, most isolates from Cameroon cluster together in one group. The majority of isolates from Latin America and the Caribbean are genetically close, but also show the tendency to cluster together by country with some exceptions. The highest genetic diversity, demonstrated by many individual clusters, was detected in the Philippines, whereas the lowest genetic diversity was found in the

Dominican Republic. No clear pattern between the genetic variation and the degree of sensitivity to DMIs was found (Figure 2).

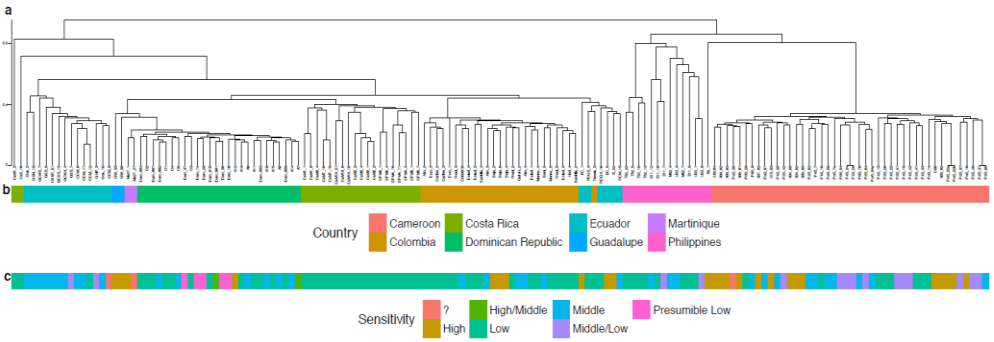

Figure 2. Genetic diversity of 155 selected *Pseudocercospora fijiensis* isolates. a) Hierarchical clustering of 155 *P. fijiensis* isolates based on 6,586 polymorphic DArTseq markers (Jaccard distance; complete linkage clustering). Classification of individual isolates based on b) the country of origin of individual isolates and by c) DMI sensitivity.

The Pfcyp51 diversity and genetic support for reduced sensitivity

Based on dissimilarities in fungicide sensitivity patterns, 266 isolates were selected for amplification and sequencing of the *Pfcyp51* gene, including the promoter region. Six wild type isolates were included as controls to determine the natural variation in *Pfcyp51* sequences irrespective of fungicide sensitivity. We identified 60 unique genotypes with a total number of 28 mutations in the coding region of the *Pfcyp51* gene (Figure 3 and Table S3) taking the sensitive strain CIRAD86 as a reference (Arango *et al.* 2016). The aa changes were dispersed over 20 positions. Strikingly, all isolates shared a nonsynonymous mutation resulting in the amino acid change V106D (Figure 3). The number of mutations per position per country is summarized in Table 3.

Table 3. Changes in the CYP51 protein sequences of *Pseudocercospora fijiensis* isolates per country.

Country/ aa subst.	Colombia	Costa Rica	Cameroon	Dom. Republic	Ecuador	Guadalupe	Philippines	Martinique	Individual Isolates	TOTAL
(n)	34	33	94	23	40	3	28	5	6	266
Promoter Insertion	24 (70.6%)	26 (78.8%)	62 (66%)	17 (74%)	5 (12%)	0	8 (28.60%)	0	0	142 (52.79%)
T18I*	34 (100%)	33 (100%)	0	23 (100%)	40 (100%)	3 (100%)	15 (53.60%)	5 (100%)	2 (33.3%)	156 (57.99%)
A19E*	1 (2.9%)	0	0	0	1 (2.5%)	0	0	0	0	2 (0.74%)
Y58F*	0	0	0	0	0	0	0	0	1 (16.6%)	1 (0.37%)
I70M	0	0	0	0	0	0	2 (7.1%)	0	0	2 (0.74%)
D71E	0	0	0	0	0	0	2 (7.1%)	0	0	2 (0.74%)
V106D*	34 (100%)	33 (100%)	94 (100%)	23 (100%)	40 (100%)	3 (100%)	28 (100%)	5 (100%)	5 (83.3%)	268 (99.63%)
V116L*	0	0	0	0	0	0	0	0	1 (16.6%)	1 (0.37%)
Y136F	21 (61.8%)	19 (57.6%)	1 (1.06%)	2 (8.70%)	0	0	4 (14.3%)	0	0	47 (17.47%)
K171R*	0	0	0	0	0	0	4 (14.3%)	0	1 (16.6%)	5 (1.86%)
V260L	0	2 (6.1%)	0	0	0	0	0	0	0	2 (0.74%)
I264T*	0	0	0	0	0	0	0	1 (20%)	0	1 (0.37%)
A313G	9 (26.5%)	19 (57.6%)	64 (68.1%)	19 (82.6%)	33 (82.5%)	0	27 (96.4%)	0	0	172 (63.94%)
H380N	0	3 (9.1%)	0	0	0	0	0	0	0	3 (1.12%)
A381G	1 (2.9%)	7 (21.2%)	0	3 (13%)	0	0	0	0	0	11 (4.09%)
R418G*	0	0	0	1 (4.3%)	0	0	0	0	0	1 (0.37%)
A446S*	0	0	0	0	0	0	22 (78.6%)	0	1 (16.7%)	23 (8.55%)
D460E	0	0	0	0	0	0	15 (53.6%)	0	0	15 (5.58%)
D460V	0	0	49 (52.1%)	0	0	0	0	0	0	49 (18.22%)
ΔY461	0	0	0	0	0	0	2 (7.1%)	0	0	2 (0.74%)
Y461D	2 (5.9%)	2 (6%)	0	0	2 (5%)	0	2 (7.1%)	0	0	8 (2.97%)
Y461N	2 (5.9%)	0	0	0	0	0	15 (53.6%)	0	0	17 (6.32%)
Y461S	0	0	0	0	0	0	2 (7.1%)	0	0	2 (0.74%)
G462A	0	1 (3%)	0	0	0	0	0	0	0	1 (0.37%)
G462D	0	0	4 (4.3%)	0	0	0	0	0	0	4 (1.49%)
Y463D	21 (61.8%)	22 (66.7%)	6 (6.4%)	14 (60.9%)	1 (2.5%)	0	6 (21.4%)	0	0	70 (26.02%)
Y463H	3 (8.8%)	1 (3%)	0	2 (8.7%)	10 (25%)	0	0	0	0	16 (5.95%)
Y463N	0	2 (6.1%)	5 (5.3%)	3 (13%)	20 (50%)	0	0	0	0	31 (11.52%)
Y463S	0	4 (12.1%)	0	2 (8.7%)	0	0	0	0	0	6 (2.23%)

*Amino acid substitutions found in sensitive isolates

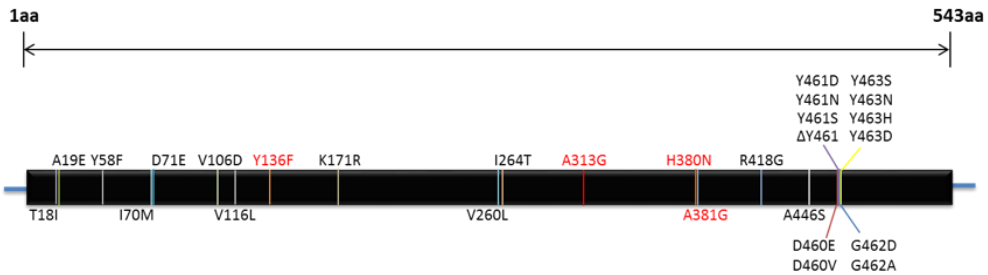

Figure 3. Amino acid (aa) substitutions identified in the *Pseudocercospora fijiensis* 14α demethylase enzyme. In total 28 aa changes were observed, located at 20 positions in the sequence of *Pfcyp51*. The substitutions with red labels are in the vicinity of the substrate recognizing site (SRS).

With the exception of Y136F, all amino acid substitutions derived from single base nonsynonymous mutations (Table S4). In Y136F, the wild type codon is TAC at position 405 bp and the altered codons are TTC and TTT, which are present in 29 isolates from different populations (Costa Rica, Cameroon, Colombia and Philippines) and in 11 isolates from Costa Rica, respectively. This may suggest that codon TTT occurred from a consecutive mutation that emerged from the pre-existing codon variant TTC. The list of the codons for each substitution is summarized in Table S4.

At a global scale, the most frequently observed aa changes are V106D (268), A313G (172), T18I (156), Y463D (70), Y136F (47) and Y461D (8) (Table 3). The largest number of specific mutations was present among Philippine isolates. Mutations resulting in I70M, D71E, D460E, ΔY461 and Y461S were unique for the Philippine population, whereas mutations leading to K171R and A446S were shared with a strain from Taiwan. However, unique mutations were also observed in other countries. For instance, V260L, H380N and G462A were exclusive for Costa Rica, whereas aa changes D460V and G462D were only found in Cameroon. Just a few mutations leading to aa changes were only found once, such as I264T in an isolate from Martinique and R418G in a strain from

the Dominican Republic (Table 3). In contrast, other mutations are ubiquitous such as T18I, present in all isolates from Latin America and the Caribbean and in 15 out of 28 Philippine isolates. The same mutation existed in two sensitive wild type strains from the Philippines and Indonesia (Table 3), but was absent among African isolates.

The number of aa changes per individual genotype varied from one to seven substitutions (Table S3). Most of the none sensitive analysed isolates gained four aa changes when compared with the reference strain. The most common combination was T18I/V106D/A313G/Y463D, present in genotypes G29 to G32, identified in 24 isolates from Colombia (2), Philippines (2), Ecuador (1), Costa Rica (5) and the Dominican Republic (14). Genotype G25, represented by one isolate from Cameroon, contained the modification Y136F (Table S3). Thirty-five isolates share only a single substitution (V106D), when compared with the CIRAD86 reference. The two and three-way combinations T18I + V106D, T18I + A19E + V106D, T18I + Y58F + V106D; T18I + V106D + I264T, T18I + V106D + R418G, T18I + V106D + A446S and, V106D + V116L + A446S were all present in *P. fijiensis* isolates sensitive to DMIs. In contrast, substitutions Y136F, A313G, H380N, D460E, D460V, ΔY461, Y461D, Y461N, Y461S, G462A, G462D, Y463D, Y463H, Y463N and Y463S were only present in strains with reduced sensitivity to DMIs. Interestingly, genotypes G8, G12, G13, G14, G18, G19, G36, G41, G49, G52, G53, G57, G58 and G60 show a differential impact on the sensitivity for the three fungicide with EC_{50} values higher on propiconazole (Table S3).

The chemical properties of the detected aa substitutions in the different genotypes are compared in Table S5. Most substitutions affect the hydrophobic or hydrophilic interactions, particularly T18I, A19E, V106D Y136F, I264T, A313G, A381G, R418G, A446S, D460V and

those at positions 461 and 463, which modulate hydrophobic or hydrophilic properties that are expected to influence the three-dimensional conformation of the protein.

Protein models and docking studies

In order to understand the conformational effect of sensitivity related substitutions on the PfCYP51 protein, seven *in silico* models were built (template base on *A. fumigatus*, *S. cerevisiae*, *H. sapiens* and *M. tuberculosis*). The quality model Z-scores are summarized in Table S6. The Z-score of a protein is defined as the energy separation between the native fold and the average of an ensemble of misfolds in the units of the standard deviation of the ensemble (Zhang & Skolnick 1998). Figure 4 shows the secondary structure of the protein model based on the CYP51 of genotype G1 (reference strain CIRAD86). The model was compared with an early *in silico* model of *P. fijiensis* and the crystal structures of the CYP51 from *Trypanosoma cruzi* and *T. brucei* (Cañas *et al.* 2009; Chen *et al.* 2010). Most of the structural CYP51 family protein elements: alpha helixes, beta sheets and the SRSs, were well conserved in the model. The exceptions are the absence of alpha helix F' and F" and the presence of an extra alpha helix predicted from aa positions 452-458. Most importantly, the SRSs were recognizable in the *in silico* PfCYP51 model and suggest an open substrate channel between the alpha helix A', the loop between alpha helix F and G (loop FG), and the loop between beta sheet 2_3 and beta sheet 2_2 (details of the active site and the channel in the model are visualized in Figure S5 and S6).

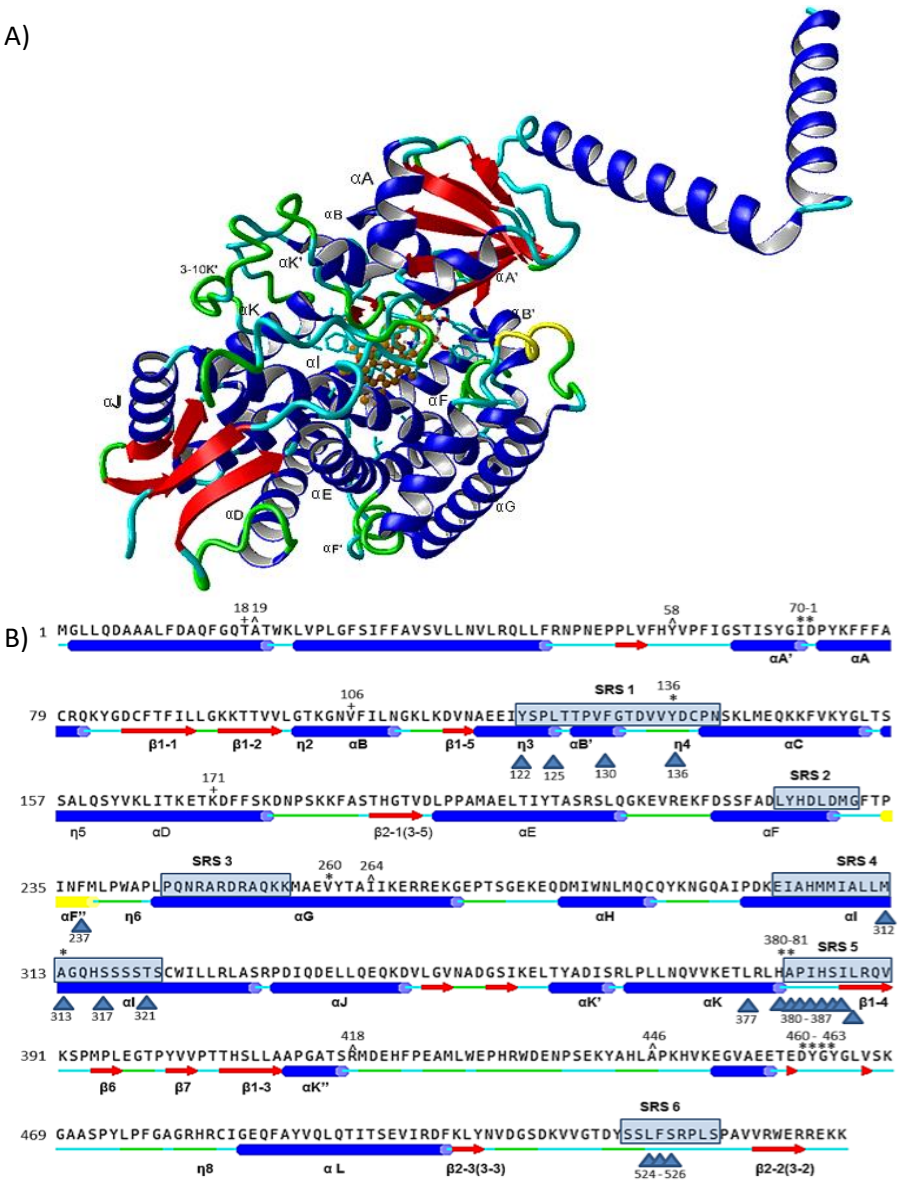

Figure 4. Schematic representations of CYP51. (A) Three-dimensional model based on *Pseudocercospora fijiensis* CIRAD86 (genotype G1). (B**)** PfCYP51 secondary structure model annotated based on Cañas et al. (2009) and Chen et al. (2010) **(**variation in nomenclature between authors is show in parentheses). Helix structures are shown as blue cylinders, ß sheets are indicated in red, turns in green and random coils in cyan. Main α helixes are depicted in capital letters and the putative substrate recognition sites (SRS) indicated as boxes. The changes in amino acids identified in *Pfcyp51* are depicted as: (^) only in DMIs sensitive isolates, (*) only in resistant strains and (+) present in both. Residues that potentially locate within 7Å of the propiconazole docking site are labelled with blue triangles.

A global distance test was performed to measure the superposition similarity between two proteins by calculating the number of structurally equivalent pairs of C-alpha atoms that are within the specified distance. This revealed that the most similar model to the PfCYP51 wild type was from the sensitive Bo_1 strain, originating from the indigenous *P. fijiensis* population in Bohol, Philippines (DMIs sensitive, genotype G10) with 85.61% of similarity, while the most dissimilar model was derived from strain CaM10_6, originating from the frequently sprayed Cartagena population in Costa Rica, (DMIs resistant, genotype G44) with 76.95% of similarity (Table S7).

Docking experiments

In silico docking experiments show that propiconazole probably binds to the PfCYP51 active site by positioning the triazole ring close to the porphyrin plane with a nitrogen atom aligned to the iron atom in the heme group (Figures 5A and S5). Based on 3D modelling putative aa positions were identified that are located less than 7Å to the nearest propiconazole atom for the docked compound. The potential interacting aa's are marked in Figure 4 and Table S7. Out of the 21 putative aa's interacting with propiconazole, 19 are located inside the proposed SRSs (Cañas *et al.* 2009). Particularly, positions 136, 313, 380 and 381 found in field isolates with reduce DMI sensitivity were predicted to be in direct interaction with propiconazole (Figure 5A). In the model of the sensitive strain Bo_1 although the amino acid substitutions were positioned outside the docking area, they induced three remarkable spatial changes in the active site chamber of the PfCYP51 (Table S7 and Figure 5B). The models of strains with reduced sensitivities revealed specific changes in the active site conformation including direct changes to some of the propiconazole interacting aa's.

All resistant models have five to eight positions with altered spatial locations and angles compared to the reference model, affecting DMI binding. Notably, the deletion at position 461 (Δ461) in model M52_10 (genotype G60, Figure 5e) results in the shift of three aa's near the docking area at positions, 524, 525 and 526. As a result, L523 is introduced into the docking site and pushes S526 to a distance of 8.13Å versus 4.05 Å in the model of sensitive strains, a distance not included in the putative range of interaction with the fungicide (Table S7). Sensitive strain Bo_1 has three positions with modulated spatial distance and angles in the PfCYP51 active site chamber (125, 380 and 384; Figure 5b).

A particular orientational variation exist at position 125, which is present in all PfCYP51 models of resistant strains, and that harbours the entrance of the channel facilitating the entry to the enzyme core that comprises the active site (Figure S6). Contrary to this conformation, the model of the sensitive strain Bo_1 has a more exposed entrance at this position while the models of the resistant strains CaM10_6, M52_10 and M52_22 have a narrow access (Figure 5b, c and f). Other major changes are situated for position 383 (reference 1.85Å close to propiconazole) and position 313. In the former case, positional changes seem to be due to aa substitutions at other positions. All predicted changes in the relative distance of aa's near the docking area (reference <7Å) are summarized in Table S7.

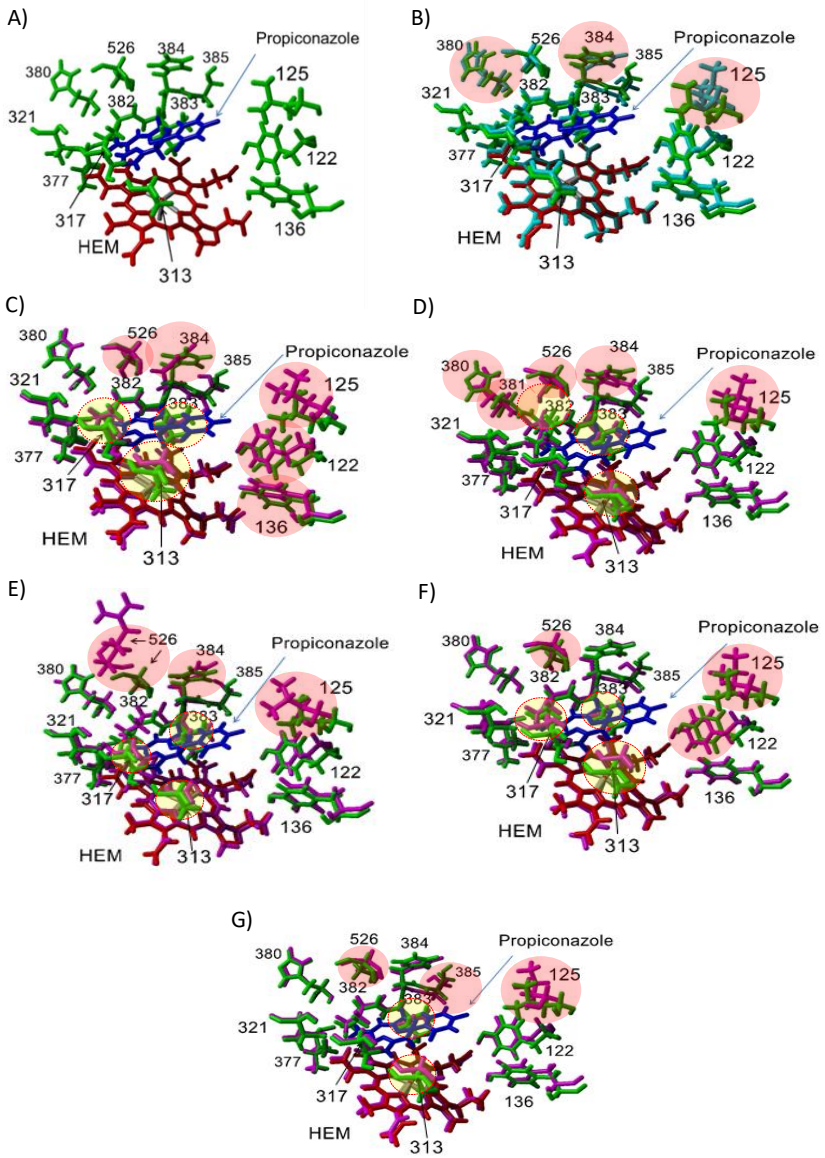

Figure 5. Models of the active site of the PfCYP51 protein with amino acid modulations due to mutations. A) Reference model of the PfCYP51 of the reference *Pseudocercospora fijiensis* CIRAD86 showing the location of amino acids (aa) in the vicinity of the propiconazole docking area. With the exception of tyrosine at position 136, aa's with a distance farther than 5.4 Å from the docking area are removed for better visualization. The heme group is depicted in red, the propiconazole fungicide in blue and aa residues in green. B) Active site of *P. fijiensis* strain Bo_1 (in cyan) superimposed on the CIRAD86 reference. Active sites of CYP51 resistant models (C) CaM10_6, (D) CaM10_21, (E) M52_10, (F) M52_22 and (G) Z4_16 (in magenta) superimposed on the CIRAD86 reference. In (D) position 381 was also included. Significant variations in distance, position or angle of the aa residuals are highlighted with light red or yellow discs.

Promoter Insertions

From the 266 sequenced isolates, we found 142 isolates that have an insertion in the *Pfcyp51* promoter (Tables 3 and S4), which have been correlated with reduced sensitivity to DMIs (Díaz-Trujillo *et al*. 2016a). For instance, the 25 *P. fijiensis* strains with the combination T18I + V106D + Y136F + Y463D (genotypes G22, G23 and G24) differed in DMI sensitivity, clearly reflected by the number of insertions in the promoters. Similarly, genotypes G35 and G36 (T18I + V106D + A313G + Y463N) do not differ in *Pfcyp51* substitutions, nonetheless, the three G36 isolates with promoter insertions have higher EC_{50} values, which maximizes in those with three insertions (Table S3).

A more detailed analysis of the promoter of the resistant strains identify a region of high variation, with insertions starting at position 2,121,774 of scaffold 7 in the genome sequence of the reference strain (*Pseudocercospora fijiensis* v2.0, JGI), ~103 bp upstream of the start codon of *Pfcyp51* (antisense direction). In 98 isolates, the insertions substitute a stretch of 8 to 27 bp starting at position 103 or 102 bp upstream of the start codon, e.g. in the Philippine isolate T52_22 an 8 bp region is substituted by an insertion of 123 bp at position -102 bp.

Others have gained multiple substitutions, such as isolate CaM3_3 from Costa Rica, which has one 16 bp exchange for a 9 bp fragment at position -103 bp and a second substitution of 7 bp with a 76 bp fragment, localized at –94 bp (Table S8). In addition, 38 isolates contain an insertion at position 94 bp. Two isolates from Cameroon, strain P2S20 and P4S19, have a substitution followed by an insertion at position 157 bp upstream of the start codon. The Philippine isolates (M52_4, M52_9, M52_23 and U22_3) show a deletion of 8 bp, “CATGGACC”, in the promoter region beginning 97 bp upstream of the start codon. Generally, the majority of insertions at the -103 region comprise one or more copies or partial

copies of a 19 bp genomic element, “TAAATCTCGTACGATAGCA”. This element is present as a single element in the CIRAD86 reference and originally located a few nucleotides downstream in the promoter MYCFIscaffold_7:2121794 – 2121813, (-122 bp upstream of the *Pfcyp51* start codon), indicated as element “A” in Figures 6 and 7.

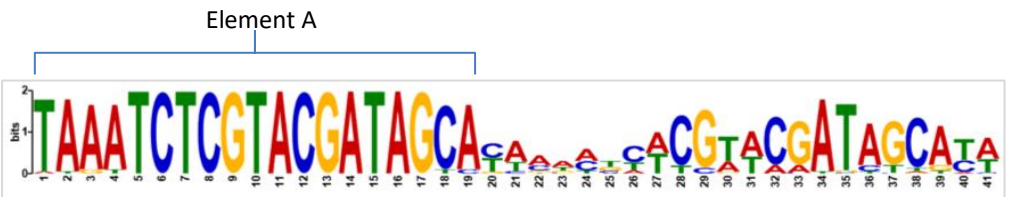

Figure 6. Logo made in MEME (Bailey & Elkan 1994) of the repeated inserts elements found in the promoter region of 142 *Pseudocercospora fijiensis* strains. Element “A” is common in all repeat candidates that were identified by the software.

Despite the geographical differences of many isolates, we identified very similar insertions in the *Pfcyp51* gene promoter. Overall, a limited number of substitutions and insertions were observed although at variable positions (Figure S7). Element “A” contains a core sequence of an eight base pair palindromic DNA fragment “TCGTACGA”, which is present in all variants, and at least twice in all isolates that contain an insertion (Figure 7; green arrows, Figure S7) and up to six copies in the *Pfcyp51* promoter of resistant strains. Some isolates contain a partial construction of element “A” in their insertions, while others have a modified “A” element due to a few additional nucleotides. For example, Philippine isolate T52_22 possesses three copies of element “A” and one partial copy, resulting in four copies of the palindrome. In a similar way, the Ecuadorian isolates RCQS_3 and RCQS_16 possess one copy of the “A” element, but three of the palindromic sequences, two of them in partial stretches of “A” (Figure S7, Table S8). In total, the palindrome sequence is present up to six

times in resistant *Pfcyp51* genotypes (Table S3). The smallest insertion, in isolate POS9 from Cameroon, encodes a single "A" element, but two copies of the palindrome (Figure 8).

The presence of two or more palindromic insertions (three or more copies in total) correlates with strongly reduced DMI sensitivity (Tables S4 and S9). Interestingly, mutation Y136F only occurred in isolates with multiple promoter insertions (at least four or more palindromes "TCGTACGA" insertions). The detailed gene configurations of representative strains with reduced sensitivity are presented in Table S3 and Figure 8.

Although geographically different isolates show very similar insertions in the *Pfcyp51* gene promoter, we found an additional big and unique insertion in Philippine isolates. This 39 bp insertion, "TTCACCACCCTCGCATTCTTGGTCA-*GTATAC*-ATAGACCT", indicated as the "B" element, is present in eight Philippine isolates (Figures 7, 8 and S5). The "B" element also encompasses a palindromic 6 bp DNA fragment "GTATAC" that, however, is not correlated with reduced sensitivity to DMIs.

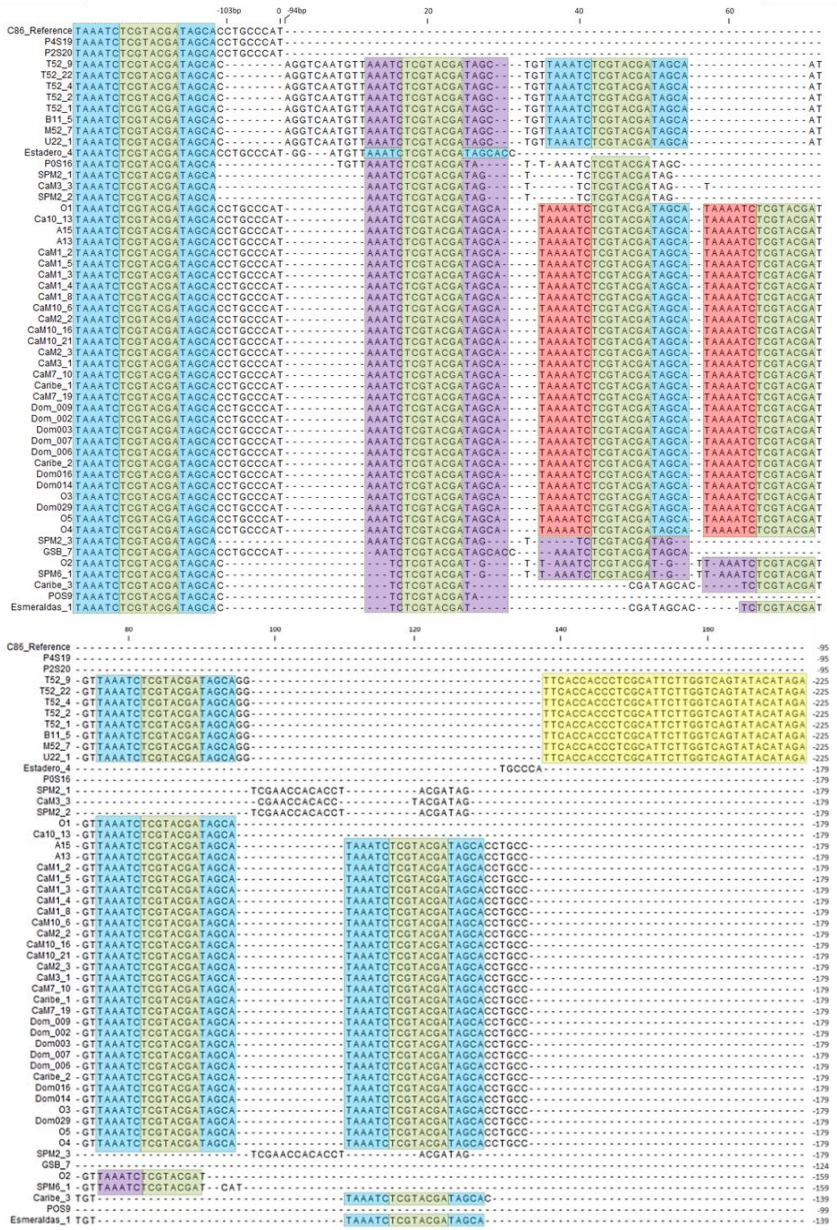

Figure 7. Analysis of the insertions in the promoter region of the *Pfcyp51* gene in *Pseudocercospora fijiensis* strains from various countries. Insertions are generally located from 94 to 103 bp upstream of the start codon of the gene. Element "A" is marked with blue together with the palindromic arrangement TCGTACGA marked in green. Alterations of element "A" are marked with red and partial constructions of the element with purple. Part of the novel insertion merely identified in Philippines isolates, element "B", is marked with light yellow. Negative values at the right represent the position from the beginning of the insertion related to the start codon of the gene.

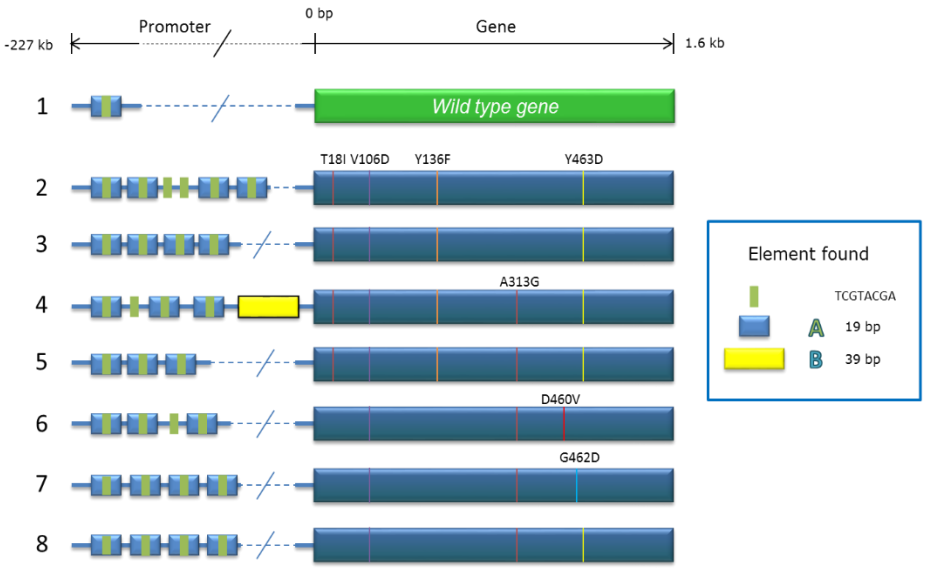

Figure 8. Representation of the *Pseudocercospora fijiensis Pfcyp51* gene. Genomic configuration of elements of the most representative resistant genotypes are shown with insertions in the promoter region of the *Pfcyp51* gene. Vertical lines in the coding domain of the *Pfcyp51* gene represent the different CYP51 codon position substitutions: 1) Reference genotype G1. 2) Resistant genotype G24. 3) Resistant genotype G23. 4) Resistant genotype G43 (Philippines). 5) Resistant genotype G42. 6) Resistant genotype G13. 7) Resistant genotype G25 and 8) Resistant genotype G18.

The effect of *Pfcyp51* mutations and fungicide sensitivity

Substitutions A313G, Y136F, H380N, Y463D and D460V gave the main explanatory changes related to increasing EC_{50} values (Table 4) as the reference genotype was a susceptible one. Additional mutation candidates for a main effect were A381G, A446S, T18I, Y463N and D460E based on a ratio of 20 for inclusion compared to the mean square error (~$p<0.00001$). However, these were less consistent, so a combination among the substitutions could be more plausible. Retaining the first 5 mutations and adding the main effect of the *Pfcyp51* promoter and the fungicide treatment resulted in even higher EC_{50} predictive power. Figure 9 shows that the number of insertions in the *Pfcyp51* promoter corresponds with reduced fungicide

sensitivity, indicated by the number after the 5 binary position representing the mutational main effect and before the fungicide letter (P). The inclusion of the fungicide factor demonstrates the main effect of the treatment but not shown in Figure 9 as the difference were too small.

Next all first order interactions were evaluated and added if significant. Followed by backward selection to check out the specific combinations that had most predictive power. Substitutions T18I, A381G, A446S are again indicated but now in combination with one or the other and a new mutation V106D is put forward in this context. Also the interaction Y136F with A313G, which are both already in the model as main effect, is still assessed as important. This combination increases again the sensitivity to the DMIs as can be seen from the parameter estimate, and seemingly this is attributed to Y136F as is also in the combination with A318G more sensitive. Finally, the addition of the promoter interaction with a mutation was all checked, however none was found to be very specific. This means that either the interaction with fungicide is already covered by a mutation or there was no specific mutation involved with the difenoconazole interaction. This last explanation is supported by the lack of significance for the alternative FW-model with sensitivities per isolate. Country is not there because the mutations are confounded with it, so country is included as last and the sensibility did not incur much from it. Figure 9 represents the effect of the accumulation of these crucial mutations by x-axis on propiconazole. In the left bottom is the sensitive reference 'without' mutation and the 'simplest' promoter of the *Pfcyp5*. The upper right has the most accumulated mutations as an additive effect together with the most insertions in the promoter that was present in the set of isolates. It shows the additive magnitude of the specific mutation combinations that was present on the $^{2}\log(EC_{50})$.

Table 4. Regression analyses of *Pseudocercospora fijiensis Pfcyp51* mutations on azole efficacy. This table shows the fitted model with the relevant factors (amino acid substitutions and promoter insertions, F-test <0.001) that remains from 23 factors evaluated. Factors are in descendant order of importance base on the accumulated analyses of the variance ratio (v.r.). The threshold of including a variable was heuristically set to a v.r. ratio of 10, which gave 11 factors as predictor for the loss of sensitivity to DMIs. This final model was checked by backward elimination to see if any previously included terms became superfluous. Table shows the degrees of freedom (d.f.), the sum of the squares (s.s), mean squares (m.s.) and variance ratio (v.r.).

Accumulated analysis of variance				
Substitution change	d.f.	s.s.	m.s.	v.r.
+ A313G	1	1876.24	1876.24	2489.04
+ Y136F	1	2268.64	2268.64	3009.60
+ H380N	1	508.66	508.66	674.79
+ Y463D	1	116.14	116.14	154.07
+ D460V	1	110.48	110.48	146.57
+ Prom	5	205.53	41.11	54.53
+ Fungi	2	64.44	32.22	42.74
+ T18I.A381G	1	51.55	51.55	68.39
+ V106D.A446S	1	148.27	148.27	196.70
+ Y136F.A313G	1	222.94	222.94	295.75
+ Y136F.A381G	1	44.60	44.60	59.17
Residual	627	472.63	0.75	
Total	643	6090.13	9.47	

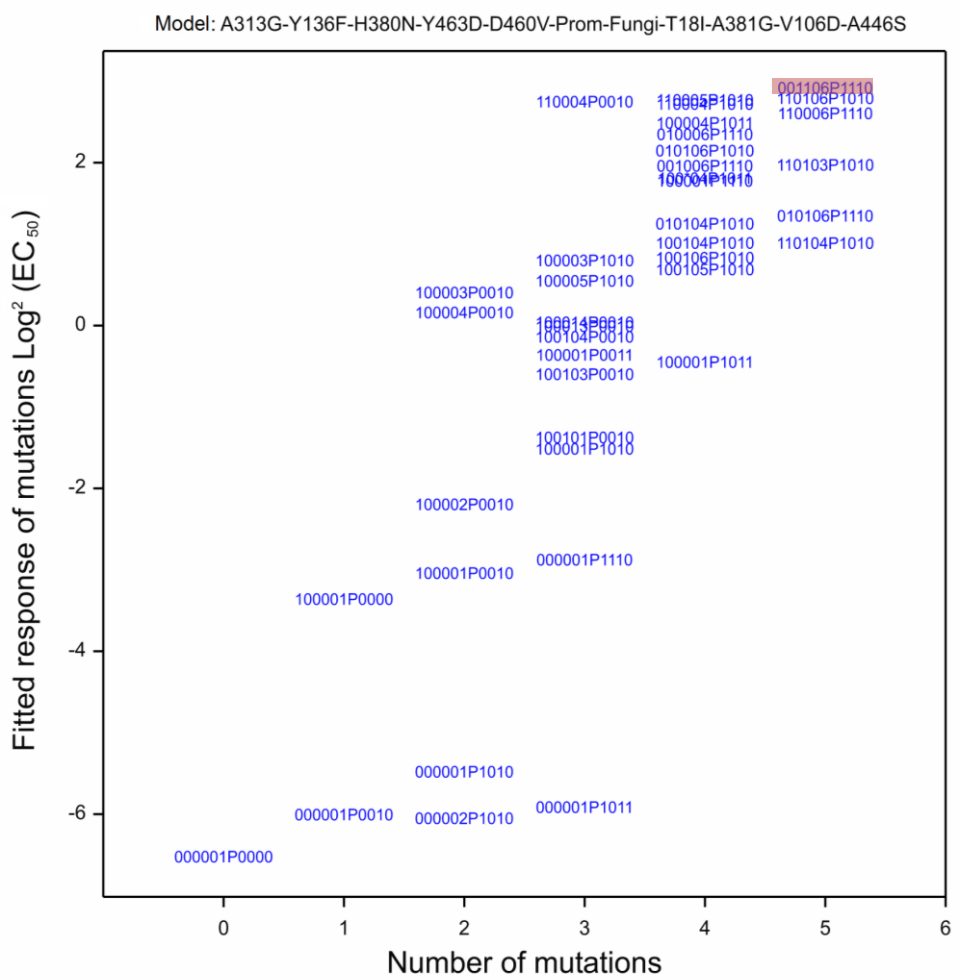

Figure 9. Predicted interaction of the accumulation of specific CYP51 substitutions with the sensitivity response on propiconazole fungicide. The genotype number codes are represented by the presence/absence of substitutions (1/0 matrix) with the exception of the *Pfcyp51* palindromic promoter insertions that have six levels. The 11 number codes follow the chosen fungicide correlated model: 1) A313G, 2) Y136F, 3) H380N, 4) Y463D, 5) D460V, 6) Promoter insert numbers, 7) Fungicide, 8) T18I, 9) A381G, 10) V106D, and 11) A446S. The substitutions are placed from left to right in order of importance where the first one is the most interactive and the last one the least interactive. For practical reasons number code 7 has been labelled for the fungicide (P for propiconazole). For example, model resistant genotype code 001106P1110 (marked in light red) has five substitutions: H380N, Y463D, T18I, A381G and V106D with six promoter palindromic inserts and it has been predicted as resistant (^{2}LogEC^{50} >0) in the interaction with the fungicide propiconazole.

Discussion

The use of antibacterial therapies and anti-fungal is common in human and veterinary medicine to complement the host immune response and restore well-being and health (Boogaerts et al. 2001). Therefore, antibiotics resistance raised global awareness (Unno et al. 2010; Yang et al. 2014), as it threatens the lives of many patients and animals due to failing antibiotic treatments, resulting in the return or severity of many bacterial infections (Brauner et al. 2016). Reduced sensitivities to fungicides equally threaten lives of patients (Eddouzi *et al.* 2013; Mitka 2011; Unno *et al.* 2010; Verweij *et al.* 2013) and animals, such as upon *Aspergillus fumigatus* infections causing aspergillosis, a lethal inflammatory disease without adequate antifungal treatment (Chowdhary *et al.* 2013; Verweij *et al.* 2013). The reduced effect of such treatments is mostly due to *cyp51* mutations (Becher & Wirsel 2012; Cools *et al.* 2013).

The control of plant pathogens also strongly relies on a limited set of fungicides, with mostly similar active ingredients (Cools *et al.* 2013). Azole fungicides are the cornerstone of contemporary managements strategies for many plant pathogens (Cools *et al.* 2013). In this paper we describe the occurrence and mechanisms of the reduced sensitivity of azole fungicides to the plant pathogenic fungus *P. fijiensis,* which may be one of the factors that leads to the increase of fungicide applications for black Sigatoka control in banana cultivation. The dispersal and magnitude of DMI fungicide resistance in *P. fijiensis* urges for an understanding of the underlying mechanisms in order to develop new control strategies. Here, we analysed an unparalleled set of *P. fijiensis* isolates obtained from populations in countries with varying practices (among them four of the top ten largest producers and exporters of banana), hence intensities of black Sigatoka management. This enables a global analysis and comparison of fungicide application and the occurrence of reduced DMI sensitivity in *P. fijiensis* and the prime genetic dynamics. The distribution of EC_{50}'s for all isolates revealed a wide range of DMI

sensitivity, parallel for the tested fungicides, which can be considered as a continuous set of values (Figure S3). This disallowed clear cut-off values to discern statistically significantly different groups. Therefore, we introduced EC_{50} criteria to form three sensitivity groups. This permitted analyses based on the non-synonymous mutations in the *Pfcyp51* gene, the promoter characteristics and the origin of the samples. Nonetheless, the result show differences in the structure of the populations based on their sensitivity response to each specific fungicide (although not significant), especially for difenoconazole. These differences may suggest the need for a better grouping criteria and differential thresholds levels choice per individual fungicide. All fungicide resistant strains were exclusively identified in commercial banana farms, especially from Costa Rica, Colombia and the Philippines, where banana production is economically very important and the number of fungicide applications per season is high (Figure 1 and Table S2).

DMI sensitivity differences are associated with fungicide application practices

Costa Rica has a long history of Sigatoka control associated with a continuously increasing number of fungicides applications per year (Chong *et al.* 2016a; Marín *et al.* 2003). For example, observed DMI baseline sensitivity shift correlates with the increasing amounts of applied fungicides, which raised from 30 in the 90's up to 50 treatments by 2007 and up to 53 in 2015 in San Pablo´s farm in Costa Rica (Chong *et al.* 2016a). In this case the actual number of DMI cycles reduced over time from seven to four applications per year in 1998 and 2014, respectively, but overall the number of DMI cycles was approximately 10 between 2003 and 2010. In 2015, there was a sudden rise in the number of DMI cycles. This rise might be resulted by the frequency of strains with high EC_{50} values in the "San Pablo" population. This event

suggests that the selective pressure in previous years was sufficient to turn the major part of the population into resistant strains by 2014. In parallel, we isolated the most resistant strains from this country and have recently proven the association between their genetic constitution and DMI sensitivity (Díaz-Trujillo *et al.* 2016a). Hence, fungicide application intensity results in the recovery of resistant strains, e.g. 99% of the Costa Rican isolates had EC_{50} values higher than 1 $mg.L^{-1}$ for propiconazole. In contrast, the majority of strains were sensitive in remote areas nearly secluded from fungicide applications. The resistant strains always carried *Pfcyp51* gene mutations. Recent findings for strobilurins suggested that these remote areas are genetically isolated from large commercial banana plantations, as indicated by their population genetic parameters (Arango *et al.* 2016). For DMIs, similar mechanisms seem to be operational. Hence, the rare occurrence of reduced sensitivity in overall sensitive populations seems to be largely due to genetic drift.

Although the actual number of DMI applications per location from which the strains were collected is in most cases untraceable it seems that the number of resistant isolates increases parallel with the number of fungicide applications (Figure 1 and Table S2) underpinning the selective pressure exerted by the intensive applications of DMIs. The relatively low percentage of resistant strains from Ecuador (difenoconazole 16.83%, epoxiconazole 8.91%, and propiconazole 21.78%) might reflect the particular climatic situation with long dry seasons at the coast, reducing black Sigatoka development and hence favouring control due to lower inoculum production. Therefore, the frequency of fungicide applications is lower (Marín *et al.* 2003), albeit that the number of applications is increasing since the sampling of the isolates in our study (2009-2010; Enrique Donoso, personal communication; CIBE, unpublished data). Hence, it would be worth monitoring the current population of *P. fijiensis* in Ecuador.

P. fijiensis incursions into Martinique and Guadalupe, two islands of the Caribbean close to the northern-east part of South America, happened only in 2010 and 2012, respectively (Guzmán *et al.* 2013; Ioos *et al.* 2011). For that reason, the exposure to the fungicide have been too short, hence the selective pressure is low, which accords with our results, as all *P. fijiensis* isolates are sensitive to DMIs. It has been found that these two populations are sensitive to other mode of action fungicide (data not shown). Thus, the favoured origin hypothesis is that these islands were colonized by wild-type *P. fijiensis* isolates. We can exclude the alternative hypothesis that the absence of continuous DMI selective pressure results in the loss of resistance alleles, due to apparent fitness costs of these alleles, which consequently reverts the population back to sensitivity. This effect was shown for *Magnaporthe oryzae* and *Cercospora beticola*, but remained unnoticed for many other fungi (Hollomon 2015), and we, therefore, consider it unlikely for *P. fijiensis*, particularly since we have identified wild-type strains in other non-sprayed areas such as San Carlos in Costa Rica and Bohol in the Philippines (data not show) and in Cameroon, Colombia and Ecuador.

P. fijiensis colonized Latin America and the Caribbean during multiple events, likely beginning around 40 years ago from Honduras and/or Costa Rica (Halkett *et al.* 2010; Lapeyre *et al.* 2010b; Rivas *et al.* 2004a). Such events are consequently accompanied by a reduction of genetic diversity through founder effects and bottleneck events (Carlier *et al.* 1996; Halkett *et al.* 2010; Hayden *et al.* 2003; Rivas *et al.* 2004b). Our results show that most isolates from Latin America and the Caribbean share the same genetic background (Figure 2). Since *P. fijiensis* ascospores cannot travel beyond a few hundred of meters, long distance dispersal is considered to be solely due to anthropogenic movement of contaminated material (Arango *et al.* 2016; Halkett *et al.* 2010; Marín *et al.* 2003; Ploetz *et al.* 2015; Rieux *et al.* 2014), which unveils

unparalleled risks for the banana sector as was also recently shown for the dissemination of the Tropical Race 4 strain of Panama disease (Ordoñez *et al.* 2015).

The genetic structure of *P. fijiensis* populations and the wild type *Pfcyp51* gene

As indicated above, most Latin American and the Caribbean *P. fijiensis* isolates cluster in the genetic analysis, while isolates from Cameroon form a distinct clade (Figure 2). Interestingly, Philippine strains show the highest diversity. This is consistent with the current understanding of the genetic structure of *P. fijiensis* populations, showing that African and American populations originate from separated colonization events and that South East Asia – here represented by the Philippines – is the centre of origin (Carlier *et al.* 1996; Halkett *et al.* 2010; Hayden *et al.* 2003; Rivas *et al.* 2004b). Intriguingly, this pattern continues at the *Pfcyp51* sequence level. For example, the substitution leading to T18I is present in all Latin American and the Caribbean and in 15 out of 34 Philippine strains, but lacks in the Cameroon population.

Our sequencing data of the *Pfcyp51* gene across all populations highlights a particularity of the CIRAD86 – originating from Cameroon - reference strain, which was selected for the first genetic linkage map and genome sequencing (Manzo-Sanchez *et al.* 2008). We now actually question the representativeness of this strain for the species as it encodes V106 in *Pfcyp51*, whereas the sequences of all 268 genotyped isolates encode D106. With the suggested centre of origin in Southeast Asia, we propose that the wild-type genotype is D106 rather than V106. In retrospect, this may indicate that the proposed additive role of V106D for DMI resistance is an artefact, based on a mutation in the hitherto reference CIRAD86. This underscores the need for more genomic information from strains that are selected in the centre of origin.

The selective pressure of DMI fungicides on *P. fijiensis*

The genetic effects of the DMI application on *P. fijiensis* populations are solely targeted on modifications of the *Pfcyp51* gene (Chong *et al.* 2016c). Most *Pfcyp51* modulations paralleled with the DMI fungicide resistance response and are comparable to those identified in other organisms. Substitutions V136A and I381V are correlated with reduced sensitivities to triadimenol in *Erysiphe necator* and to tebuconazole in *Zymoceptoria. tritici*, respectively (Cools *et al.* 2013). The accumulation of mutations tend to confer increased resistance to DMI fungicides (Cools *et al.* 2013). Here, we were unable to determine such specific substitutions for any of the tested fungicides, which might be due to the high number of factors analysed (individual mutations, mutation combination and seven levels of promoter insertions) and hence, further studies may identify unique mutation/efficacy interactions.

Sensitive strains also show variation in *Pfcyp51* with a maximum of three mutations resulting in three aa changes. Overall, the maximum of aa substitutions was found in the Philippines population where some isolates accumulated up to seven aa substitutions in the coding region of the *Pfcyp51* gene. Such a high degree of polymorphism in the *cyp51* gene was previously reported for *Oculimacula (Tapesia) acuformis* and *Oculimacula yallundae* (Albertini *et al.* 2003). The substitutions resulting in A19E, I70M, D71E, V260L, I264T, H380N, R418G, D460E, D460V, Y461N, Y461S, ΔY461 and G462D were hitherto unknown in *P. fijiensis*, although other changes in positions 461 and 462 were reported to affect DMI sensitivity (Cañas *et al.* 2009; Chong *et al.* 2010; Díaz-Trujillo *et al.* 2016a). Substitutions A19E, Y58F, V116L, and R418G were solely detected in DMI sensitive isolates, suggesting that these represent natural random variation, which is uncorrelated with DMI sensitivity. Notably, substitution I264T - although also detected in a DMI sensitive isolate (EC_{50} slightly above sensitive mean) - was correlated with additive effects of reduced efficacy of the evaluated DMIs. Similarly,

substitutions T18I and A446S are present in both sensitive and resistant isolates, but also correlated with additive effects in strains with reduced sensitivity. These observed additive effects might be explained as compensatory substitutions for azole sensitivity as illustrated by aa changes at positions 459 to 461 in ZtCYP51, compensating the I381V substitution that was, by itself, enzymatically lethal as corroborated by complementation experiments in *S. cerevisiae* (Becher & Wirsel 2012). Nevertheless, these modifications urge for additional studies to elucidate their contribution to *P. fijiensis* survival.

Substitutions A313G, Y136F, H380N, Y463D, and D460V are directly correlated with resistance (Table 4 and Figure 9). Similar substitutions were also found in *Z. tritici* (Cools *et al.* 2013) and Y136F was linked with azole resistance in *Penicillium italicum*, *Uncinula necator* and *Blumeria graminis* f. sp. *hordei* (Albertini *et al.* 2003; Délye *et al.* 1997). A substitution at Y136, or its equivalent in other species, is the most frequently observed modification of CYP51 in pathogenic fungi (Cools *et al.* 2013). Interestingly, Y136F originated from two sequential codons. The original codon is TAC while the modified codons are TTC and TTT. The latter is unique for the Costa Rican population and might arose from a consecutive mutation emerging from the pre-existing TTC codon. This consecutive selection might result from prolonged DMI pressure and may represent a bias event towards optimized codon usage. Nonetheless it is worth to mention that *P. fijiensis* codon usage shows a relative preference for codon TTC (http://www.kazusa.or.jp/codon/). Codon usage in genes have been long investigated in *Echericha coli*, *Sacharomices cerevisiae and Aspergillus nidulans* where it correlated with highly expressed genes and more efficient translation (Dilucca *et al.* 2015; Lloyd & Sharp 1991; Trotta 2013). The *Pfcyp51* gene overexpression in Costa Rican isolations (Chong *et al.* 2010) (Díaz-Trujillo *et al.* 2016a) might supports this hypothesis, however additional studies are needed to strengthen this hypothesis.

The importance of substitutions at positions 136, 313, 380, 381 and 460 to 463 are strengthened by PfCYP51 modelling. Everything is located in the SRS with the exception of positions 460 to 463. Changes in these aa positions however compromise the three-dimensional structure of the protein resulting in an affinity change. For example, models with the setting ΔY461, Y461N, G462A and Y463D, revealed significant distance and angle changes around position 524 to 526 (SRS6) (Figure 4, 5c - g, Table S7). The deletion of ΔY461 itself provoked a shift in positions 523 to 526 introducing the S523 into the active site and pushing S526 out of the selected range (>7Å).

Position 125, at the entrance of the channel to the active site of the protein, was modified in all resistant strains (Figure S6). However, based on modelling, the effect is limited. Additional studies are required to elucidate how these changes affect fungicide entry or the catalytic centre structure.

Promoter insertions

The presence of repeated elements and insertions in the promoter region of *Pfcyp51* explains the overexpression of the gene (Díaz-Trujillo *et al.* 2016a). None of the sensitive strains contained insertions while they were very common in tolerant and resistant strains. In the current survey, promoter insertions positively correlated with the resistance to DMIs (Table 4 and Figure 9). Also in *A. fumigatus*, *cyp51* promoter insertions explain resistance to azole fungicides (Mellado *et al.* 2007). Interestingly, these insertions were also associated with non-synonymous mutations in the coding region (Mellado *et al.* 2007). Snelders et al. (2012) observed that an *A. fumigatus* isolate with two copies of a tandem repeat acquired an additional repeat during DMI treatment, supporting the hypothesis that genomic changes in the *cyp51* gene are inducible

(Snelders *et al.* 2012). Analogously, in *P. digitatum* promoter insertions drive the expression of the *cyp51* gene; a 126 bp insertion comprising five repeat elements is present in resistant strains while sensitive isolates only carry one repeat element (Hamamoto *et al.* 2000). Similarly, *cyp51* gene overexpression is also reported in *Z. tritici*, a close relative of *P. fijiensis*, where a 120 bp insertion in the promoter region correlates with a 10 to 40-fold overexpression (Cools *et al.* 2012) as well as in *Venturia inaequalis* and *Blumeriella jaapii* where the presence of upstream derivatives of LINE-like retrotransposons correlated with overexpression of the *cyp51* gene (Ma *et al.* 2006; Schnabel & Jones 2000). All these discussed inserts vary in size and nature across species and are not located at equal positions and clearly result from independent events, which raise the question about their origin. They might be remains of transposable element activity, some of which contain powerful promoters (Cools *et al.* 2013). In *P. digitatum* the effect of a transposon element in the promoter region has been described to confer resistance to DMIs (Sun *et al.* 2013). In *P. fijiensis* three independent promoter insertions exist, at -103 bp, at -94 bp and at -157 bp from the start codon. The latter was only present in two isolates from Cameroon. However, all isolates with insertion contain tandem copies (or partial copies) of the "A" element and were at least DMIs "tolerant" (>0.1 $mg.L^{-1}$) (Table S3 and Figure S7).

The central core of the repeats are the palindromic arrangements. These motifs constitute an important group of regulatory elements in eukaryotes in which they act as cis-elements (Knox & Keller 2015). Many transcription factors (TF) bind palindromic sequences with high affinity (Narlikar & Hartemink 2006; Qian *et al.* 2006). For example, the TF ADR1 binds as a monomer to palindromic sequences to regulate the expression of *S. cerevisiae ADH2* gene (Thurkral *et al.* 1991). In *Cercospora nicotianae* the TF CRG1 binds to a palindrome sequence present in genes that confer resistance to cercosporin (Chung *et al.* 2003). The group of bZIP TFs target palindromic DNA sequences as dimers, thereby regulating e.g. secondary metabolism (Knox & Keller 2015). The importance of the palindromic sequences might explain

the existence of isolates with few full repeats and a partial "A" element insertion in *Pfcyp51* while they are categorized as DMI resistant (Figure 7 and S5).

A second palindromic sequence, inserted in element B, was present in the *Pfcyp51* promoter of Philippine isolates. Due to the absence of intermediate strains, only containing the B element, the correlation with *Pfcyp51* gene expression is not resolved. However, there was no significant expression difference of *Pfcyp51* when compared with strains merely containing the A element (data not shown).

In summary, element "A" and particularly its palindromic core is important for regulation of gene expression, most likely as a transcriptional enhancer (Bolton *et al.* 2016; Schnabel & Jones 2000). The mechanism and the components involved, however, remain to be elucidated. Future work will aim at the characterization of the mechanism and identification of the involved TFs and additional determinants (Bolton *et al.* 2016). Promoter insertions of element A tend to confer higher EC_{50} regardless of the fungicide and might be the reason why we were unable to determine specific substitutions for the tested fungicides. This might suggest that the effect of the promoter insertion can mascaraed the specific interaction between a substitution and a particular fungicide and induce at some degree cross-resistance among DMI fungicides. Interestingly, only tolerant or resistant strains show insertions in the promoter region. This suggest that the selection for overexpression only occur after the emergence of point mutations. Transformation studies have demonstrated that insertions alone do not increase the DMI resistant significantly (Díaz-Trujillo *et al.* 2016a). For this reason, we conclude that the main resistant factors are the mutations in the *Pfcyp51* and that the insertions in the promoter region acts as an additive effect.

Three isolates from Costa Rica, CaM10_6, CaM1_5 and CaM3_1, revealed extraordinary high EC_{50} values that remain unexplained solely by the *Pfcyp51* promoter

configuration similar to those in other, less resistant isolates from Costa Rica. This suggests the presence of additional quantitative genetic components that directly or indirectly modulate resistance as observed in *O. yallundae* (Dyer *et al.* 2000). The construction of a genetic map of *P. fijiensis* based on crosses between fungicide resistant and sensitive isolates facilitates an unbiased identification of additional genes contributing to DMI fungicide resistance (Chong *et al.* 2016c) and provides insight into the recombination frequency of mutant alleles and the possible distribution mechanism of resistance alleles in populations. The current and associated studies (Chong *et al.* 2016a; Chong *et al.* 2016c; Díaz-Trujillo *et al.* 2016a) significantly contribute to the understanding of the origin and dissemination of DMI resistance mechanisms in *P. fijiensis* and facilitates the prediction of the efficacy of new generations of fungicides.

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Supporting information

Table S1. EC_{50} mean values per *Pseudocercospora fijiensis* isolate. Columns show: Country, isolate code, fungicide, 2 Logarithmic mean, lower and upper error of the difference values (Lsed and Used), observations (number of independent EC_{50} calculated values), standard error of the measurement (Sem), lower and upper confident intervals of the means and the back-transformed EC_{50} mean values in $mg.L^{-1}$. Strains with EC_{50} values lower than 0.1 $mg.L^{-1}$ are indicated with a green background, values from 0.1 to 0.9 $mg.L^{-1}$are shown in light yellow background and values higher than 1 $mg.L^{-1}$ are shown in light red background.

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Dominican Rep.	A_10	Difenoconazole	-3.525	-5.567	-1.482	3	0.520	-4.546	-2.504	**0.087**
Dominican Rep.	A_10	Epoxiconazole	-4.238	-6.280	-2.195	3	0.520	-5.259	-3.217	**0.053**
Dominican Rep.	A_10	Propiconazole	-2.484	-4.527	-0.442	3	0.520	-3.506	-1.463	**0.179**
Dominican Rep.	A_11	Difenoconazole	0.437	-1.606	2.479	3	0.520	-0.584	1.458	**1.354**
Dominican Rep.	A_11	Epoxiconazole	-0.057	-2.100	1.985	3	0.520	-1.079	0.964	**0.961**
Dominican Rep.	A_11	Propiconazole	0.309	-1.733	2.352	3	0.520	-0.712	1.330	**1.239**
Dominican Rep.	A_12	Difenoconazole	-7.164	-9.207	-5.122	3	0.520	-8.185	-6.143	**0.007**
Dominican Rep.	A_12	Epoxiconazole	-6.334	-8.376	-4.292	3	0.520	-7.355	-5.313	**0.012**
Dominican Rep.	A_12	Propiconazole	-5.956	-7.999	-3.914	3	0.520	-6.978	-4.935	**0.016**
Dominican Rep.	A_13	Difenoconazole	1.082	-0.960	3.124	3	0.520	0.061	2.103	**2.117**
Dominican Rep.	A_13	Epoxiconazole	-0.107	-2.149	1.936	3	0.520	-1.128	0.915	**0.929**
Dominican Rep.	A_13	Propiconazole	0.153	-1.889	2.196	3	0.520	-0.868	1.174	**1.112**
Dominican Rep.	A_14	Difenoconazole	-1.781	-3.824	0.261	3	0.520	-2.802	-0.760	**0.291**
Dominican Rep.	A_14	Epoxiconazole	-2.274	-4.317	-0.232	3	0.520	-3.295	-1.253	**0.207**
Dominican Rep.	A_14	Propiconazole	-1.780	-3.822	0.262	3	0.520	-2.801	-0.759	**0.291**
Dominican Rep.	A_15	Difenoconazole	0.877	-1.165	2.920	3	0.520	-0.144	1.899	**1.837**
Dominican Rep.	A_15	Epoxiconazole	0.221	-1.822	2.263	3	0.520	-0.801	1.242	**1.165**
Dominican Rep.	A_15	Propiconazole	0.650	-1.393	2.692	3	0.520	-0.372	1.671	**1.569**
Dominican Rep.	A_16	Difenoconazole	-4.152	-6.194	-2.110	3	0.520	-5.173	-3.131	**0.056**
Dominican Rep.	A_16	Epoxiconazole	-2.032	-4.074	0.011	3	0.520	-3.053	-1.010	**0.245**
Dominican Rep.	A_16	Propiconazole	-1.632	-3.674	0.410	3	0.520	-2.653	-0.611	**0.323**
Dominican Rep.	A_7	Difenoconazole	-2.646	-4.689	-0.604	3	0.520	-3.667	-1.625	**0.160**
Dominican Rep.	A_7	Epoxiconazole	-4.329	-6.371	-2.287	3	0.520	-5.350	-3.308	**0.050**
Dominican Rep.	A_7	Propiconazole	-1.882	-3.924	0.160	3	0.520	-2.903	-0.861	**0.271**
Dominican Rep.	A_8	Difenoconazole	-2.057	-4.100	-0.015	3	0.520	-3.079	-1.036	**0.240**
Dominican Rep.	A_8	Epoxiconazole	-3.478	-5.521	-1.436	3	0.520	-4.500	-2.457	**0.090**
Dominican Rep.	A_8	Propiconazole	-1.459	-3.501	0.583	3	0.520	-2.480	-0.438	**0.364**
Dominican Rep.	A_9	Difenoconazole	-1.630	-3.672	0.412	3	0.520	-2.651	-0.609	**0.323**
Dominican Rep.	A_9	Epoxiconazole	-2.694	-4.736	-0.651	3	0.520	-3.715	-1.673	**0.155**
Dominican Rep.	A_9	Propiconazole	-1.278	-3.321	0.764	3	0.520	-2.300	-0.257	**0.412**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Colombia	Almendros_1	Difenoconazole	0.809	-1.233	2.852	1	0.901	-0.959	2.578	**1.752**
Colombia	Almendros_1	Epoxiconazole	-0.412	-2.454	1.630	1	0.901	-2.181	1.357	**0.752**
Colombia	Almendros_1	Propiconazole	1.061	-0.982	3.103	1	0.901	-0.708	2.830	**2.086**
Colombia	Almendros_2	Difenoconazole	2.569	0.527	4.612	1	0.901	0.801	4.338	**5.936**
Colombia	Almendros_2	Epoxiconazole	0.237	-1.806	2.279	1	0.901	-1.532	2.006	**1.178**
Colombia	Almendros_2	Propiconazole	1.581	-0.461	3.624	1	0.901	-0.187	3.350	**2.993**
Colombia	Almendros_3	Difenoconazole	-0.173	-2.215	1.869	1	0.901	-1.942	1.596	**0.887**
Colombia	Almendros_3	Epoxiconazole	-0.896	-2.939	1.146	1	0.901	-2.665	0.872	**0.537**
Colombia	Almendros_3	Propiconazole	-0.267	-2.310	1.775	1	0.901	-2.036	1.502	**0.831**
Colombia	Almendros_4	Difenoconazole	-1.589	-3.632	0.453	1	0.901	-3.358	0.180	**0.332**
Colombia	Almendros_4	Epoxiconazole	-1.093	-3.135	0.949	1	0.901	-2.862	0.676	**0.469**
Colombia	Almendros_4	Propiconazole	-0.406	-2.448	1.637	1	0.901	-2.174	1.363	**0.755**
Colombia	Almendros_8	Difenoconazole	2.898	0.855	4.940	1	0.901	1.129	4.666	**7.452**
Colombia	Almendros_8	Epoxiconazole	2.672	0.630	4.715	1	0.901	0.904	4.441	**6.375**
Colombia	Almendros_8	Propiconazole	2.759	0.716	4.801	1	0.901	0.990	4.528	**6.768**
Philippines	B11_10	Difenoconazole	0.645	-1.397	2.688	1	0.901	-1.123	2.414	**1.564**
Philippines	B11_10	Epoxiconazole	1.071	-0.972	3.113	1	0.901	-0.698	2.839	**2.100**
Philippines	B11_10	Propiconazole	1.600	-0.442	3.642	1	0.901	-0.169	3.369	**3.031**
Philippines	B11_11	Difenoconazole	-0.976	-3.018	1.066	3	0.520	-1.997	0.045	**0.508**
Philippines	B11_11	Epoxiconazole	-2.089	-4.131	-0.046	3	0.520	-3.110	-1.067	**0.235**
Philippines	B11_11	Propiconazole	-0.542	-2.585	1.500	3	0.520	-1.564	0.479	**0.687**
Philippines	B11_12	Difenoconazole	-0.836	-2.878	1.207	3	0.520	-1.857	0.186	**0.560**
Philippines	B11_12	Epoxiconazole	-1.531	-3.574	0.511	3	0.520	-2.552	-0.510	**0.346**
Philippines	B11_12	Propiconazole	-0.264	-2.306	1.778	3	0.520	-1.285	0.757	**0.833**
Philippines	B11_13	Difenoconazole	-1.458	-3.501	0.584	3	0.520	-2.480	-0.437	**0.364**
Philippines	B11_13	Epoxiconazole	-1.909	-3.952	0.133	3	0.520	-2.930	-0.888	**0.266**
Philippines	B11_13	Propiconazole	0.187	-1.856	2.229	3	0.520	-0.835	1.208	**1.138**
Philippines	B11_14	Difenoconazole	-1.566	-3.609	0.476	1	0.901	-3.335	0.202	**0.338**
Philippines	B11_14	Epoxiconazole	-1.599	-3.641	0.444	1	0.901	-3.367	0.170	**0.330**
Philippines	B11_14	Propiconazole	-0.067	-2.110	1.975	1	0.901	-1.836	1.701	**0.954**
Philippines	B11_15	Difenoconazole	1.128	-0.914	3.170	1	0.901	-0.641	2.897	**2.186**
Philippines	B11_15	Epoxiconazole	0.639	-1.403	2.682	1	0.901	-1.129	2.408	**1.558**
Philippines	B11_15	Propiconazole	0.952	-1.091	2.994	1	0.901	-0.817	2.720	**1.934**
Philippines	B11_16	Difenoconazole	0.325	-1.718	2.367	1	0.901	-1.444	2.094	**1.252**
Philippines	B11_16	Epoxiconazole	-1.187	-3.229	0.856	1	0.901	-2.955	0.582	**0.439**
Philippines	B11_16	Propiconazole	1.260	-0.782	3.302	1	0.901	-0.509	3.029	**2.395**
Philippines	B11_2	Difenoconazole	1.317	-0.725	3.360	1	0.901	-0.452	3.086	**2.492**
Philippines	B11_2	Epoxiconazole	1.781	-0.262	3.823	1	0.901	0.012	3.550	**3.436**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Philippines	B11_2	Propiconazole	2.175	0.132	4.217	1	0.901	0.406	3.943	**4.514**
Philippines	B11_5	Difenoconazole	2.456	0.414	4.498	3	0.520	1.435	3.477	**5.487**
Philippines	B11_5	Epoxiconazole	2.184	0.142	4.227	3	0.520	1.163	3.205	**4.545**
Philippines	B11_5	Propiconazole	2.122	0.080	4.164	3	0.520	1.101	3.143	**4.353**
Philippines	B11_7	Difenoconazole	-0.487	-2.530	1.555	3	0.520	-1.508	0.534	**0.713**
Philippines	B11_7	Epoxiconazole	-1.627	-3.669	0.416	3	0.520	-2.648	-0.606	**0.324**
Philippines	B11_7	Propiconazole	0.121	-1.922	2.163	3	0.520	-0.901	1.142	**1.087**
Philippines	B11_8	Difenoconazole	1.506	-0.536	3.549	1	0.901	-0.262	3.275	**2.841**
Philippines	B11_8	Epoxiconazole	1.355	-0.688	3.397	1	0.901	-0.414	3.124	**2.558**
Philippines	B11_8	Propiconazole	1.355	-0.687	3.397	1	0.901	-0.414	3.124	**2.558**
Philippines	B11_9	Difenoconazole	0.571	-1.471	2.613	1	0.901	-1.198	2.340	**1.486**
Philippines	B11_9	Epoxiconazole	-0.401	-2.444	1.641	1	0.901	-2.170	1.367	**0.757**
Philippines	B11_9	Propiconazole	0.218	-1.825	2.260	1	0.901	-1.551	1.987	**1.163**
Philippines	B21_1	Difenoconazole	-0.795	-2.837	1.248	1	0.901	-2.564	0.974	**0.576**
Philippines	B21_1	Epoxiconazole	-0.480	-2.522	1.563	1	0.901	-2.248	1.289	**0.717**
Philippines	B21_1	Propiconazole	1.335	-0.708	3.377	1	0.901	-0.434	3.103	**2.522**
Philippines	B21_10	Difenoconazole	0.410	-1.633	2.452	1	0.901	-1.359	2.179	**1.328**
Philippines	B21_10	Epoxiconazole	-0.132	-2.175	1.910	1	0.901	-1.901	1.637	**0.912**
Philippines	B21_10	Propiconazole	1.586	-0.456	3.628	1	0.901	-0.183	3.355	**3.002**
Philippines	B21_11	Difenoconazole	1.088	-0.954	3.131	1	0.901	-0.680	2.857	**2.126**
Philippines	B21_11	Epoxiconazole	0.598	-1.444	2.641	1	0.901	-1.171	2.367	**1.514**
Philippines	B21_11	Propiconazole	0.974	-1.068	3.017	1	0.901	-0.795	2.743	**1.965**
Philippines	B21_12	Difenoconazole	0.855	-1.187	2.897	1	0.901	-0.914	2.624	**1.809**
Philippines	B21_12	Epoxiconazole	1.847	-0.195	3.889	1	0.901	0.078	3.616	**3.598**
Philippines	B21_12	Propiconazole	-1.016	-3.058	1.027	1	0.901	-2.785	0.753	**0.495**
Philippines	B21_13	Difenoconazole	-1.097	-3.139	0.945	1	0.901	-2.866	0.672	**0.467**
Philippines	B21_13	Epoxiconazole	-3.184	-5.227	-1.142	1	0.901	-4.953	-1.415	**0.110**
Philippines	B21_13	Propiconazole	0.204	-1.838	2.246	1	0.901	-1.565	1.973	**1.152**
Philippines	B21_2	Difenoconazole	0.041	-2.001	2.084	1	0.901	-1.727	1.810	**1.029**
Philippines	B21_2	Epoxiconazole	0.317	-1.726	2.359	1	0.901	-1.452	2.086	**1.246**
Philippines	B21_2	Propiconazole	1.185	-0.858	3.227	1	0.901	-0.584	2.953	**2.273**
Philippines	B21_3	Difenoconazole	-0.213	-2.255	1.830	1	0.901	-1.981	1.556	**0.863**
Philippines	B21_3	Epoxiconazole	-0.838	-2.880	1.204	1	0.901	-2.607	0.931	**0.559**
Philippines	B21_3	Propiconazole	0.664	-1.379	2.706	1	0.901	-1.105	2.432	**1.584**
Philippines	B21_4	Difenoconazole	2.864	0.821	4.906	1	0.901	1.095	4.633	**7.279**
Philippines	B21_4	Epoxiconazole	2.970	0.928	5.012	1	0.901	1.201	4.739	**7.836**
Philippines	B21_4	Propiconazole	0.592	-1.451	2.634	1	0.901	-1.177	2.360	**1.507**
Philippines	B21_5	Difenoconazole	-2.006	-4.049	0.036	1	0.901	-3.775	-0.237	**0.249**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Philippines	B21_5	Epoxiconazole	-1.278	-3.321	0.764	1	0.901	-3.047	0.490	**0.412**
Philippines	B21_5	Propiconazole	-0.108	-2.150	1.935	1	0.901	-1.877	1.661	**0.928**
Philippines	B21_6	Difenoconazole	-0.241	-2.283	1.802	1	0.901	-2.009	1.528	**0.846**
Philippines	B21_6	Epoxiconazole	1.012	-1.031	3.054	1	0.901	-0.757	2.780	**2.016**
Philippines	B21_6	Propiconazole	-0.207	-2.250	1.835	1	0.901	-1.976	1.561	**0.866**
Philippines	B21_7	Difenoconazole	1.021	-1.022	3.063	1	0.901	-0.748	2.789	**2.029**
Philippines	B21_7	Epoxiconazole	1.025	-1.017	3.068	1	0.901	-0.743	2.794	**2.036**
Philippines	B21_7	Propiconazole	-0.068	-2.111	1.974	1	0.901	-1.837	1.700	**0.954**
Philippines	B21_8	Difenoconazole	1.965	-0.077	4.008	1	0.901	0.196	3.734	**3.905**
Philippines	B21_8	Epoxiconazole	2.255	0.213	4.298	1	0.901	0.486	4.024	**4.774**
Philippines	B21_8	Propiconazole	0.747	-1.296	2.789	1	0.901	-1.022	2.515	**1.678**
Philippines	B21_9	Difenoconazole	2.421	0.378	4.463	1	0.901	0.652	4.190	**5.354**
Philippines	B21_9	Epoxiconazole	1.236	-0.806	3.279	1	0.901	-0.533	3.005	**2.356**
Philippines	B21_9	Propiconazole	2.528	0.486	4.570	1	0.901	0.759	4.297	**5.768**
Colombia	Bananal_1	Difenoconazole	0.703	-1.340	2.745	1	0.901	-1.066	2.472	**1.628**
Colombia	Bananal_1	Epoxiconazole	0.660	-1.382	2.703	1	0.901	-1.108	2.429	**1.580**
Colombia	Bananal_1	Propiconazole	1.185	-0.857	3.228	1	0.901	-0.584	2.954	**2.274**
Colombia	Bejuquillo_1	Difenoconazole	-6.750	-8.792	-4.707	1	0.901	-8.519	-4.981	**0.009**
Colombia	Bejuquillo_1	Epoxiconazole	-6.087	-8.130	-4.045	1	0.901	-7.856	-4.318	**0.015**
Colombia	Bejuquillo_1	Propiconazole	-5.946	-7.989	-3.904	1	0.901	-7.715	-4.177	**0.016**
Colombia	Bejuquillo_2	Difenoconazole	-6.561	-8.603	-4.518	3	0.520	-7.582	-5.539	**0.011**
Colombia	Bejuquillo_2	Epoxiconazole	-6.057	-8.099	-4.015	3	0.520	-7.078	-5.036	**0.015**
Colombia	Bejuquillo_2	Propiconazole	-5.236	-7.278	-3.194	3	0.520	-6.257	-4.215	**0.027**
Colombia	Bejuquillo_3	Difenoconazole	-5.921	-7.963	-3.878	1	0.901	-7.689	-4.152	**0.017**
Colombia	Bejuquillo_3	Epoxiconazole	-5.129	-7.172	-3.087	1	0.901	-6.898	-3.360	**0.029**
Colombia	Bejuquillo_3	Propiconazole	-3.874	-5.916	-1.832	1	0.901	-5.643	-2.105	**0.068**
Colombia	Bejuquillo_4	Difenoconazole	-7.447	-9.490	-5.405	1	0.901	-9.216	-5.679	**0.006**
Colombia	Bejuquillo_4	Epoxiconazole	-6.075	-8.118	-4.033	1	0.901	-7.844	-4.307	**0.015**
Colombia	Bejuquillo_4	Propiconazole	-5.606	-7.648	-3.564	1	0.901	-7.375	-3.837	**0.021**
Colombia	Bejuquillo_5	Difenoconazole	-6.075	-8.117	-4.032	1	0.901	-7.843	-4.306	**0.015**
Colombia	Bejuquillo_5	Epoxiconazole	-5.805	-7.847	-3.763	1	0.901	-7.574	-4.036	**0.018**
Colombia	Bejuquillo_5	Propiconazole	-4.304	-6.346	-2.262	1	0.901	-6.073	-2.535	**0.051**
Colombia	Bejuquillo_6	Difenoconazole	-6.266	-8.308	-4.224	1	0.901	-8.035	-4.497	**0.013**
Colombia	Bejuquillo_6	Epoxiconazole	-5.937	-7.980	-3.895	1	0.901	-7.706	-4.168	**0.016**
Colombia	Bejuquillo_6	Propiconazole	-4.865	-6.907	-2.822	1	0.901	-6.634	-3.096	**0.034**
Colombia	Bejuquillo_7	Difenoconazole	-6.158	-8.200	-4.116	1	0.901	-7.927	-4.389	**0.014**
Colombia	Bejuquillo_7	Epoxiconazole	-5.323	-7.366	-3.281	1	0.901	-7.092	-3.554	**0.025**
Colombia	Bejuquillo_7	Propiconazole	-4.963	-7.005	-2.920	1	0.901	-6.731	-3.194	**0.032**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Philippines	Bo_1	Difenoconazole	-7.483	-9.525	-5.441	3	0.520	-8.504	-6.462	**0.006**
Philippines	Bo_1	Epoxiconazole	-6.447	-8.490	-4.405	3	0.520	-7.468	-5.426	**0.011**
Philippines	Bo_1	Propiconazole	-5.851	-7.893	-3.808	3	0.520	-6.872	-4.829	**0.017**
Colombia	Bonita_2	Difenoconazole	-2.089	-4.131	-0.047	3	0.520	-3.110	-1.068	**0.235**
Colombia	Bonita_2	Epoxiconazole	-2.749	-4.791	-0.706	3	0.520	-3.770	-1.727	**0.149**
Colombia	Bonita_2	Propiconazole	-1.796	-3.839	0.246	3	0.520	-2.817	-0.775	**0.288**
Colombia	C080910	Difenoconazole	-3.023	-5.066	-0.981	3	0.520	-4.044	-2.002	**0.123**
Colombia	C080910	Epoxiconazole	-3.082	-5.125	-1.040	3	0.520	-4.103	-2.061	**0.118**
Colombia	C080910	Propiconazole	-1.178	-3.221	0.864	3	0.520	-2.199	-0.157	**0.442**
Colombia	C120901	Difenoconazole	-1.591	-3.633	0.452	1	0.901	-3.359	0.178	**0.332**
Colombia	C120901	Epoxiconazole	-2.594	-4.636	-0.552	1	0.901	-4.363	-0.825	**0.166**
Colombia	C120901	Propiconazole	-1.061	-3.104	0.981	1	0.901	-2.830	0.708	**0.479**
Colombia	C120906	Difenoconazole	2.761	0.719	4.804	1	0.901	0.993	4.530	**6.781**
Colombia	C120906	Epoxiconazole	3.072	1.030	5.115	1	0.901	1.304	4.841	**8.411**
Colombia	C120906	Propiconazole	2.924	0.881	4.966	1	0.901	1.155	4.692	**7.587**
Colombia	C120908	Difenoconazole	2.764	0.722	4.807	1	0.901	0.995	4.533	**6.794**
Colombia	C120908	Epoxiconazole	1.580	-0.462	3.623	1	0.901	-0.189	3.349	**2.990**
Colombia	C120908	Propiconazole	1.313	-0.729	3.355	1	0.901	-0.456	3.082	**2.485**
Colombia	C120909	Difenoconazole	2.568	0.525	4.610	1	0.901	0.799	4.336	**5.928**
Colombia	C120909	Epoxiconazole	0.394	-1.648	2.437	1	0.901	-1.375	2.163	**1.314**
Colombia	C120909	Propiconazole	2.464	0.422	4.507	1	0.901	0.696	4.233	**5.519**
Colombia	C120910	Difenoconazole				0				**>10.24**
Colombia	C120910	Epoxiconazole				0				**>10.24**
Colombia	C120910	Propiconazole				0				**>10.24**
Colombia	C120912	Difenoconazole	2.585	0.543	4.628	1	0.901	0.816	4.354	**6.001**
Colombia	C120912	Epoxiconazole	-0.191	-2.234	1.851	1	0.901	-1.960	1.578	**0.876**
Colombia	C120912	Propiconazole	1.799	-0.244	3.841	1	0.901	0.030	3.568	**3.479**
Colombia	C120913	Difenoconazole				0				**>10.24**
Colombia	C120913	Epoxiconazole	2.597	0.555	4.640	1	0.901	0.828	4.366	**6.051**
Colombia	C120913	Propiconazole				0				**>10.24**
Colombia	C139	Difenoconazole	-6.430	-8.473	-4.388	2	0.637	-7.681	-5.179	**0.012**
Colombia	C139	Epoxiconazole	-6.116	-8.158	-4.074	2	0.637	-7.367	-4.865	**0.014**
Colombia	C139	Propiconazole	-5.387	-7.429	-3.344	2	0.637	-6.638	-4.136	**0.024**
Cameroon	C86	Difenoconazole	-7.862	-9.905	-5.820	2	0.637	-9.113	-6.612	**0.004**
Cameroon	C86	Epoxiconazole	-7.217	-9.259	-5.175	2	0.637	-8.468	-5.966	**0.007**
Cameroon	C86	Propiconazole	-6.681	-8.723	-4.638	2	0.637	-7.932	-5.430	**0.010**
Costa Rica	Ca10_13	Difenoconazole	2.486	0.444	4.529	2	0.637	1.236	3.737	**5.604**
Costa Rica	Ca10_13	Epoxiconazole	1.759	-0.283	3.801	2	0.637	0.508	3.010	**3.385**

Country	Isolate	Fungicide	[2]Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Costa Rica	Ca10_13	Propiconazole	1.990	-0.053	4.032	2	0.637	0.739	3.240	**3.971**
Costa Rica	Ca5_16	Difenoconazole	2.489	0.446	4.531	2	0.637	1.238	3.739	**5.613**
Costa Rica	Ca5_16	Epoxiconazole	0.328	-1.715	2.370	2	0.637	-0.923	1.578	**1.255**
Costa Rica	Ca5_16	Propiconazole	1.473	-0.569	3.515	2	0.637	0.222	2.724	**2.776**
Costa Rica	CaM1_1	Difenoconazole	2.583	0.541	4.625	7	0.341	1.914	3.252	**5.992**
Costa Rica	CaM1_1	Epoxiconazole	0.890	-1.153	2.932	6	0.368	0.168	1.612	**1.853**
Costa Rica	CaM1_1	Propiconazole	1.443	-0.600	3.485	7	0.341	0.774	2.111	**2.718**
Costa Rica	CaM1_10	Difenoconazole	2.263	0.221	4.305	4	0.451	1.379	3.147	**4.800**
Costa Rica	CaM1_10	Epoxiconazole	2.155	0.112	4.197	4	0.451	1.270	3.039	**4.453**
Costa Rica	CaM1_10	Propiconazole	1.974	-0.069	4.016	3	0.520	0.953	2.995	**3.928**
Costa Rica	CaM1_11	Difenoconazole	1.472	-0.571	3.514	2	0.637	0.221	2.722	**2.774**
Costa Rica	CaM1_11	Epoxiconazole	2.144	0.101	4.186	2	0.637	0.893	3.395	**4.419**
Costa Rica	CaM1_11	Propiconazole	1.702	-0.341	3.744	2	0.637	0.451	2.952	**3.253**
Costa Rica	CaM1_12	Difenoconazole	3.296	1.253	5.338	1	0.901	1.527	5.064	**9.819**
Costa Rica	CaM1_12	Epoxiconazole	1.695	-0.348	3.737	1	0.901	-0.074	3.464	**3.237**
Costa Rica	CaM1_12	Propiconazole	2.357	0.315	4.399	2	0.637	1.106	3.608	**5.123**
Costa Rica	CaM1_13	Difenoconazole				0				**>10.24**
Costa Rica	CaM1_13	Epoxiconazole				0				**>10.24**
Costa Rica	CaM1_13	Propiconazole				0				**>10.24**
Costa Rica	CaM1_14	Difenoconazole	2.732	0.690	4.775	1	0.901	0.964	4.501	**6.645**
Costa Rica	CaM1_14	Epoxiconazole	1.758	-0.285	3.800	1	0.901	-0.011	3.527	**3.382**
Costa Rica	CaM1_14	Propiconazole	1.966	-0.076	4.008	1	0.901	0.197	3.735	**3.907**
Costa Rica	CaM1_15	Difenoconazole				0				**>10.24**
Costa Rica	CaM1_15	Epoxiconazole	1.904	-0.138	3.947	1	0.901	0.135	3.673	**3.743**
Costa Rica	CaM1_15	Propiconazole				0				**>10.24**
Costa Rica	CaM1_16	Difenoconazole				0				**>10.24**
Costa Rica	CaM1_16	Epoxiconazole	3.002	0.960	5.044	2	0.637	1.751	4.253	**8.012**
Costa Rica	CaM1_16	Propiconazole	2.952	0.910	4.995	1	0.901	1.183	4.721	**7.739**
Costa Rica	CaM1_2	Difenoconazole	2.031	-0.011	4.074	5	0.403	1.240	2.822	**4.088**
Costa Rica	CaM1_2	Epoxiconazole	1.515	-0.527	3.558	7	0.341	0.847	2.184	**2.859**
Costa Rica	CaM1_2	Propiconazole	2.503	0.460	4.545	5	0.403	1.712	3.294	**5.668**
Costa Rica	CaM1_3	Difenoconazole	2.429	0.386	4.471	6	0.368	1.707	3.151	**5.384**
Costa Rica	CaM1_3	Epoxiconazole	1.896	-0.146	3.938	7	0.341	1.228	2.565	**3.722**
Costa Rica	CaM1_3	Propiconazole	1.793	-0.250	3.835	7	0.341	1.124	2.461	**3.464**
Costa Rica	CaM1_4	Difenoconazole	1.646	-0.397	3.688	7	0.341	0.977	2.314	**3.129**
Costa Rica	CaM1_4	Epoxiconazole	1.957	-0.085	4.000	6	0.368	1.235	2.679	**3.883**
Costa Rica	CaM1_4	Propiconazole	2.215	0.173	4.258	6	0.368	1.493	2.937	**4.644**
Costa Rica	CaM1_5	Difenoconazole	3.706	1.664	5.749	3	0.520	2.685	4.728	**13.054**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Costa Rica	CaM1_5	Epoxiconazole	2.372	0.329	4.414	4	0.451	1.487	3.256	**5.176**
Costa Rica	CaM1_5	Propiconazole	2.630	0.588	4.673	5	0.403	1.839	3.421	**6.191**
Costa Rica	CaM1_6	Difenoconazole	2.256	0.214	4.299	5	0.403	1.465	3.047	**4.778**
Costa Rica	CaM1_6	Epoxiconazole	2.225	0.182	4.267	5	0.403	1.434	3.016	**4.675**
Costa Rica	CaM1_6	Propiconazole	2.609	0.567	4.652	5	0.403	1.818	3.400	**6.103**
Costa Rica	CaM1_7	Difenoconazole	2.348	0.305	4.390	6	0.368	1.626	3.070	**5.091**
Costa Rica	CaM1_7	Epoxiconazole	2.654	0.612	4.697	6	0.368	1.932	3.376	**6.296**
Costa Rica	CaM1_7	Propiconazole	2.550	0.508	4.592	5	0.403	1.759	3.341	**5.856**
Costa Rica	CaM1_8	Difenoconazole	1.479	-0.563	3.522	6	0.368	0.757	2.201	**2.788**
Costa Rica	CaM1_8	Epoxiconazole	2.151	0.108	4.193	6	0.368	1.429	2.873	**4.440**
Costa Rica	CaM1_8	Propiconazole	0.949	-1.093	2.992	6	0.368	0.227	1.671	**1.931**
Costa Rica	CaM1_9	Difenoconazole	1.943	-0.099	3.986	2	0.637	0.693	3.194	**3.846**
Costa Rica	CaM1_9	Epoxiconazole	1.616	-0.427	3.658	1	0.901	-0.153	3.385	**3.065**
Costa Rica	CaM1_9	Propiconazole	2.568	0.526	4.611	2	0.637	1.318	3.819	**5.931**
Costa Rica	CaM10_16	Difenoconazole	2.588	0.546	4.631	3	0.520	1.567	3.610	**6.014**
Costa Rica	CaM10_16	Epoxiconazole	1.215	-0.828	3.257	3	0.520	0.193	2.236	**2.321**
Costa Rica	CaM10_16	Propiconazole	2.488	0.445	4.530	3	0.520	1.467	3.509	**5.609**
Costa Rica	CaM10_21	Difenoconazole	2.961	0.919	5.003	3	0.520	1.940	3.982	**7.787**
Costa Rica	CaM10_21	Epoxiconazole	3.330	1.287	5.372	1	0.901	1.561	5.098	**10.054**
Costa Rica	CaM10_21	Propiconazole	2.655	0.613	4.697	3	0.520	1.634	3.676	**6.299**
Costa Rica	CaM10_6	Difenoconazole	4.387	2.345	6.430	3	0.520	3.366	5.408	**20.925**
Costa Rica	CaM10_6	Epoxiconazole	2.655	0.613	4.697	6	0.368	1.933	3.377	**6.298**
Costa Rica	CaM10_6	Propiconazole	4.203	2.160	6.245	1	0.901	2.434	5.971	**18.414**
Costa Rica	CaM2_1	Difenoconazole	2.400	0.357	4.442	3	0.520	1.378	3.421	**5.277**
Costa Rica	CaM2_1	Epoxiconazole	1.324	-0.719	3.366	2	0.637	0.073	2.574	**2.503**
Costa Rica	CaM2_1	Propiconazole	1.264	-0.779	3.306	4	0.451	0.379	2.148	**2.401**
Costa Rica	CaM2_10	Difenoconazole				0				**>10.24**
Costa Rica	CaM2_10	Epoxiconazole	2.759	0.717	4.802	2	0.637	1.509	4.010	**6.770**
Costa Rica	CaM2_10	Propiconazole	2.874	0.832	4.917	2	0.637	1.624	4.125	**7.333**
Costa Rica	CaM2_11	Difenoconazole				0				**>10.24**
Costa Rica	CaM2_11	Epoxiconazole				0				**>10.24**
Costa Rica	CaM2_11	Propiconazole				0				**>10.24**
Costa Rica	CaM2_12	Difenoconazole				0				**>10.24**
Costa Rica	CaM2_12	Epoxiconazole				0				**>10.24**
Costa Rica	CaM2_12	Propiconazole				0				**>10.24**
Costa Rica	CaM2_13	Difenoconazole				0				**>10.24**
Costa Rica	CaM2_13	Epoxiconazole				0				**>10.24**
Costa Rica	CaM2_13	Propiconazole				0				**>10.24**

Country	Isolate	Fungicide	[2]Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Costa Rica	CaM2_14	Difenoconazole				0				**>10.24**
Costa Rica	CaM2_14	Epoxiconazole	2.784	0.742	4.827	2	0.637	1.534	4.035	**6.889**
Costa Rica	CaM2_14	Propiconazole	3.004	0.961	5.046	2	0.637	1.753	4.254	**8.020**
Costa Rica	CaM2_15	Difenoconazole	2.072	0.029	4.114	1	0.901	0.303	3.840	**4.204**
Costa Rica	CaM2_15	Epoxiconazole	1.939	-0.104	3.981	2	0.637	0.688	3.190	**3.834**
Costa Rica	CaM2_15	Propiconazole	2.337	0.294	4.379	2	0.637	1.086	3.587	**5.051**
Costa Rica	CaM2_16	Difenoconazole	3.255	1.213	5.298	1	0.901	1.487	5.024	**9.549**
Costa Rica	CaM2_16	Epoxiconazole	2.785	0.743	4.827	2	0.637	1.534	4.036	**6.892**
Costa Rica	CaM2_16	Propiconazole	2.737	0.694	4.779	2	0.637	1.486	3.988	**6.666**
Costa Rica	CaM2_2	Difenoconazole	3.015	0.972	5.057	2	0.637	1.764	4.265	**8.082**
Costa Rica	CaM2_2	Epoxiconazole	2.072	0.030	4.115	2	0.637	0.822	3.323	**4.205**
Costa Rica	CaM2_2	Propiconazole	2.022	-0.020	4.065	3	0.520	1.001	3.044	**4.063**
Costa Rica	CaM2_3	Difenoconazole	2.315	0.273	4.358	2	0.637	1.065	3.566	**4.977**
Costa Rica	CaM2_3	Epoxiconazole	2.349	0.306	4.391	3	0.520	1.328	3.370	**5.094**
Costa Rica	CaM2_3	Propiconazole	2.046	0.004	4.088	4	0.451	1.162	2.930	**4.130**
Costa Rica	CaM2_4	Difenoconazole	2.839	0.796	4.881	3	0.520	1.817	3.860	**7.153**
Costa Rica	CaM2_4	Epoxiconazole	2.204	0.161	4.246	3	0.520	1.183	3.225	**4.607**
Costa Rica	CaM2_4	Propiconazole	2.678	0.636	4.720	3	0.520	1.657	3.699	**6.400**
Costa Rica	CaM2_5	Difenoconazole				0				**>10.24**
Costa Rica	CaM2_5	Epoxiconazole	2.843	0.800	4.885	1	0.901	1.074	4.611	**7.173**
Costa Rica	CaM2_5	Propiconazole	2.915	0.872	4.957	1	0.901	1.146	4.684	**7.541**
Costa Rica	CaM2_6	Difenoconazole				0				**>10.24**
Costa Rica	CaM2_6	Epoxiconazole	2.402	0.359	4.444	1	0.901	0.633	4.171	**5.285**
Costa Rica	CaM2_6	Propiconazole	2.765	0.723	4.807	1	0.901	0.996	4.534	**6.797**
Costa Rica	CaM2_7	Difenoconazole	1.905	-0.138	3.947	1	0.901	0.136	3.674	**3.745**
Costa Rica	CaM2_7	Epoxiconazole	1.798	-0.245	3.840	2	0.637	0.547	3.048	**3.476**
Costa Rica	CaM2_7	Propiconazole	1.990	-0.052	4.033	2	0.637	0.740	3.241	**3.974**
Costa Rica	CaM2_8	Difenoconazole				0				**>10.24**
Costa Rica	CaM2_8	Epoxiconazole				0				**>10.24**
Costa Rica	CaM2_8	Propiconazole				0				**>10.24**
Costa Rica	CaM2_9	Difenoconazole	2.632	0.590	4.674	2	0.637	1.381	3.883	**6.199**
Costa Rica	CaM2_9	Epoxiconazole	2.138	0.095	4.180	2	0.637	0.887	3.388	**4.401**
Costa Rica	CaM2_9	Propiconazole	2.946	0.903	4.988	2	0.637	1.695	4.197	**7.705**
Costa Rica	CaM3_1	Difenoconazole	4.228	2.186	6.270	3	0.520	3.207	5.249	**18.741**
Costa Rica	CaM3_1	Epoxiconazole	1.941	-0.101	3.983	6	0.368	1.219	2.663	**3.840**
Costa Rica	CaM3_1	Propiconazole	3.050	1.007	5.092	5	0.403	2.259	3.841	**8.280**
Costa Rica	CaM3_10	Difenoconazole				0				**>10.24**
Costa Rica	CaM3_10	Epoxiconazole	2.611	0.568	4.653	1	0.901	0.842	4.379	**6.108**

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Costa Rica	CaM3_10	Propiconazole	3.302	1.260	5.345	1	0.901	1.533	5.071	**9.864**
Costa Rica	CaM3_11	Difenoconazole	2.236	0.194	4.279	2	0.637	0.986	3.487	**4.712**
Costa Rica	CaM3_11	Epoxiconazole	1.216	-0.827	3.258	2	0.637	-0.035	2.466	**2.322**
Costa Rica	CaM3_11	Propiconazole	2.764	0.722	4.807	2	0.637	1.514	4.015	**6.794**
Costa Rica	CaM3_12	Difenoconazole	2.739	0.697	4.782	1	0.901	0.971	4.508	**6.678**
Costa Rica	CaM3_12	Epoxiconazole	0.716	-1.326	2.759	2	0.637	-0.534	1.967	**1.643**
Costa Rica	CaM3_12	Propiconazole	2.179	0.137	4.222	2	0.637	0.929	3.430	**4.529**
Costa Rica	CaM3_13	Difenoconazole	2.831	0.788	4.873	1	0.901	1.062	4.599	**7.114**
Costa Rica	CaM3_13	Epoxiconazole	2.801	0.758	4.843	2	0.637	1.550	4.051	**6.967**
Costa Rica	CaM3_13	Propiconazole	3.057	1.014	5.099	1	0.901	1.288	4.825	**8.320**
Costa Rica	CaM3_14	Difenoconazole	1.248	-0.794	3.291	1	0.901	-0.520	3.017	**2.376**
Costa Rica	CaM3_14	Epoxiconazole	2.289	0.246	4.331	2	0.637	1.038	3.539	**4.886**
Costa Rica	CaM3_14	Propiconazole	2.215	0.172	4.257	1	0.901	0.446	3.983	**4.642**
Costa Rica	CaM3_15	Difenoconazole				0				**>10.24**
Costa Rica	CaM3_15	Epoxiconazole	1.692	-0.350	3.735	2	0.637	0.441	2.943	**3.231**
Costa Rica	CaM3_15	Propiconazole				0				**>10.24**
Costa Rica	CaM3_16	Difenoconazole	2.869	0.827	4.911	2	0.637	1.618	4.120	**7.305**
Costa Rica	CaM3_16	Epoxiconazole	2.133	0.090	4.175	2	0.637	0.882	3.383	**4.386**
Costa Rica	CaM3_16	Propiconazole	2.627	0.585	4.670	2	0.637	1.377	3.878	**6.179**
Costa Rica	CaM3_2	Difenoconazole	2.392	0.350	4.435	2	0.637	1.142	3.643	**5.250**
Costa Rica	CaM3_2	Epoxiconazole	1.526	-0.516	3.569	3	0.520	0.505	2.547	**2.880**
Costa Rica	CaM3_2	Propiconazole	2.092	0.049	4.134	4	0.451	1.207	2.976	**4.263**
Costa Rica	CaM3_3	Difenoconazole	1.679	-0.364	3.721	3	0.520	0.658	2.700	**3.202**
Costa Rica	CaM3_3	Epoxiconazole	0.022	-2.020	2.065	4	0.451	-0.862	0.907	**1.016**
Costa Rica	CaM3_3	Propiconazole	1.477	-0.566	3.519	4	0.451	0.592	2.361	**2.783**
Costa Rica	CaM3_4	Difenoconazole	3.145	1.103	5.188	1	0.901	1.376	4.914	**8.847**
Costa Rica	CaM3_4	Epoxiconazole	1.705	-0.337	3.747	4	0.451	0.821	2.589	**3.261**
Costa Rica	CaM3_4	Propiconazole	3.126	1.084	5.169	3	0.520	2.105	4.148	**8.732**
Costa Rica	CaM3_5	Difenoconazole				0				**>10.24**
Costa Rica	CaM3_5	Epoxiconazole	2.075	0.033	4.118	2	0.637	0.824	3.326	**4.214**
Costa Rica	CaM3_5	Propiconazole	3.190	1.147	5.232	1	0.901	1.421	4.958	**9.123**
Costa Rica	CaM3_6	Difenoconazole				0				**>10.24**
Costa Rica	CaM3_6	Epoxiconazole	3.095	1.052	5.137	1	0.901	1.326	4.864	**8.543**
Costa Rica	CaM3_6	Propiconazole				0				**>10.24**
Costa Rica	CaM3_7	Difenoconazole	1.739	-0.303	3.781	2	0.637	0.488	2.990	**3.338**
Costa Rica	CaM3_7	Epoxiconazole	1.467	-0.576	3.509	2	0.637	0.216	2.717	**2.764**
Costa Rica	CaM3_7	Propiconazole	0.726	-1.316	2.769	2	0.637	-0.525	1.977	**1.654**
Costa Rica	CaM3_8	Difenoconazole	2.078	0.036	4.121	2	0.637	0.828	3.329	**4.223**

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Costa Rica	CaM3_8	Epoxiconazole	1.687	-0.355	3.730	2	0.637	0.437	2.938	**3.221**
Costa Rica	CaM3_8	Propiconazole	1.583	-0.459	3.626	2	0.637	0.333	2.834	**2.997**
Costa Rica	CaM3_9	Difenoconazole	1.729	-0.313	3.771	2	0.637	0.478	2.980	**3.315**
Costa Rica	CaM3_9	Epoxiconazole	0.329	-1.714	2.371	2	0.637	-0.922	1.579	**1.256**
Costa Rica	CaM3_9	Propiconazole	0.931	-1.111	2.974	2	0.637	-0.320	2.182	**1.907**
Costa Rica	CaM7_10	Difenoconazole	2.833	0.791	4.875	3	0.520	1.812	3.854	**7.126**
Costa Rica	CaM7_10	Epoxiconazole	2.284	0.242	4.327	3	0.520	1.263	3.305	**4.871**
Costa Rica	CaM7_10	Propiconazole	1.114	-0.929	3.156	3	0.520	0.092	2.135	**2.164**
Costa Rica	CaM7_19	Difenoconazole	2.595	0.553	4.638	2	0.637	1.344	3.846	**6.042**
Costa Rica	CaM7_19	Epoxiconazole	2.141	0.098	4.183	3	0.520	1.119	3.162	**4.410**
Costa Rica	CaM7_19	Propiconazole	2.342	0.299	4.384	3	0.520	1.320	3.363	**5.069**
Colombia	Caribe_1	Difenoconazole	2.901	0.858	4.943	1	0.901	1.132	4.670	**7.468**
Colombia	Caribe_1	Epoxiconazole	2.435	0.393	4.478	1	0.901	0.667	4.204	**5.409**
Colombia	Caribe_1	Propiconazole	2.774	0.731	4.816	1	0.901	1.005	4.543	**6.839**
Colombia	Caribe_2	Difenoconazole	1.603	-0.439	3.646	3	0.520	0.582	2.624	**3.038**
Colombia	Caribe_2	Epoxiconazole	0.771	-1.272	2.813	3	0.520	-0.250	1.792	**1.706**
Colombia	Caribe_2	Propiconazole	0.195	-1.847	2.237	3	0.520	-0.826	1.216	**1.145**
Colombia	Caribe_3	Difenoconazole	2.510	0.467	4.552	3	0.520	1.488	3.531	**5.694**
Colombia	Caribe_3	Epoxiconazole	1.322	-0.720	3.364	3	0.520	0.301	2.343	**2.500**
Colombia	Caribe_3	Propiconazole	1.669	-0.373	3.712	3	0.520	0.648	2.690	**3.181**
Colombia	Caribe_4	Difenoconazole	0.205	-1.838	2.247	1	0.901	-1.564	1.973	**1.152**
Colombia	Caribe_4	Epoxiconazole	-1.105	-3.147	0.938	1	0.901	-2.873	0.664	**0.465**
Colombia	Caribe_4	Propiconazole	-0.271	-2.313	1.772	1	0.901	-2.039	1.498	**0.829**
Dominican Rep.	Dom_1	Difenoconazole	-3.777	-5.820	-1.735	3	0.520	-4.799	-2.756	**0.073**
Dominican Rep.	Dom_1	Epoxiconazole	-5.681	-7.723	-3.638	3	0.520	-6.702	-4.660	**0.019**
Dominican Rep.	Dom_1	Propiconazole	-2.374	-4.417	-0.332	3	0.520	-3.396	-1.353	**0.193**
Dominican Rep.	Dom_10	Difenoconazole	0.779	-1.264	2.821	3	0.520	-0.243	1.800	**1.716**
Dominican Rep.	Dom_10	Epoxiconazole	0.863	-1.179	2.905	3	0.520	-0.158	1.884	**1.819**
Dominican Rep.	Dom_10	Propiconazole	1.242	-0.801	3.284	3	0.520	0.221	2.263	**2.365**
Dominican Rep.	Dom_12	Difenoconazole	-2.578	-4.620	-0.535	3	0.520	-3.599	-1.557	**0.168**
Dominican Rep.	Dom_12	Epoxiconazole	-2.366	-4.408	-0.324	3	0.520	-3.387	-1.345	**0.194**
Dominican Rep.	Dom_12	Propiconazole	-0.324	-2.366	1.718	3	0.520	-1.345	0.697	**0.799**
Dominican Rep.	Dom_2	Difenoconazole	0.068	-1.975	2.110	3	0.520	-0.953	1.089	**1.048**
Dominican Rep.	Dom_2	Epoxiconazole	-0.024	-2.067	2.018	3	0.520	-1.045	0.997	**0.983**
Dominican Rep.	Dom_2	Propiconazole	-0.207	-2.249	1.835	3	0.520	-1.228	0.814	**0.866**
Dominican Rep.	Dom_3	Difenoconazole	-0.151	-2.194	1.891	3	0.520	-1.172	0.870	**0.901**
Dominican Rep.	Dom_3	Epoxiconazole	-0.250	-2.293	1.792	3	0.520	-1.272	0.771	**0.841**
Dominican Rep.	Dom_3	Propiconazole	0.403	-1.639	2.445	3	0.520	-0.618	1.424	**1.322**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Dominican Rep.	Dom_4	Difenoconazole	0.325	-1.718	2.367	3	0.520	-0.697	1.346	**1.252**
Dominican Rep.	Dom_4	Epoxiconazole	0.346	-1.696	2.389	3	0.520	-0.675	1.367	**1.271**
Dominican Rep.	Dom_4	Propiconazole	0.855	-1.188	2.897	3	0.520	-0.166	1.876	**1.808**
Dominican Rep.	Dom_6	Difenoconazole	-0.950	-2.992	1.093	3	0.520	-1.971	0.072	**0.518**
Dominican Rep.	Dom_6	Epoxiconazole	-0.615	-2.657	1.428	3	0.520	-1.636	0.406	**0.653**
Dominican Rep.	Dom_6	Propiconazole	0.053	-1.989	2.095	3	0.520	-0.968	1.074	**1.037**
Dominican Rep.	Dom_7	Difenoconazole	0.264	-1.778	2.307	3	0.520	-0.757	1.285	**1.201**
Dominican Rep.	Dom_7	Epoxiconazole	0.277	-1.766	2.319	3	0.520	-0.745	1.298	**1.211**
Dominican Rep.	Dom_7	Propiconazole	0.561	-1.482	2.603	3	0.520	-0.460	1.582	**1.475**
Dominican Rep.	Dom_8	Difenoconazole	-1.161	-3.204	0.881	3	0.520	-2.183	-0.140	**0.447**
Dominican Rep.	Dom_8	Epoxiconazole	-0.824	-2.866	1.218	3	0.520	-1.845	0.197	**0.565**
Dominican Rep.	Dom_8	Propiconazole	-0.009	-2.052	2.033	3	0.520	-1.030	1.012	**0.994**
Dominican Rep.	Dom_9	Difenoconazole	-0.163	-2.206	1.879	3	0.520	-1.184	0.858	**0.893**
Dominican Rep.	Dom_9	Epoxiconazole	-0.542	-2.585	1.500	3	0.520	-1.564	0.479	**0.687**
Dominican Rep.	Dom_9	Propiconazole	0.180	-1.862	2.222	3	0.520	-0.841	1.201	**1.133**
Ecuador	E_22	Difenoconazole	-7.618	-9.660	-5.575	4	0.451	-8.502	-6.733	**0.005**
Ecuador	E_22	Epoxiconazole	-6.926	-8.968	-4.883	3	0.520	-7.947	-5.904	**0.008**
Ecuador	E_22	Propiconazole	-6.565	-8.608	-4.523	5	0.403	-7.356	-5.774	**0.011**
Ecuador	EC_1	Difenoconazole	-7.756	-9.798	-5.714	4	0.451	-8.640	-6.872	**0.005**
Ecuador	EC_1	Epoxiconazole	-6.993	-9.036	-4.951	3	0.520	-8.014	-5.972	**0.008**
Ecuador	EC_1	Propiconazole	-6.062	-8.104	-4.020	4	0.451	-6.946	-5.178	**0.015**
Ecuador	EC_21	Difenoconazole	-7.897	-9.940	-5.855	1	0.901	-9.666	-6.128	**0.004**
Ecuador	EC_21	Epoxiconazole	-7.651	-9.694	-5.609	1	0.901	-9.420	-5.882	**0.005**
Ecuador	EC_21	Propiconazole	-6.748	-8.790	-4.706	1	0.901	-8.517	-4.979	**0.009**
Ecuador	EC_5	Difenoconazole	-7.084	-9.126	-5.041	4	0.451	-7.968	-6.199	**0.007**
Ecuador	EC_5	Epoxiconazole	-6.813	-8.855	-4.771	4	0.451	-7.697	-5.929	**0.009**
Ecuador	EC_5	Propiconazole	-6.470	-8.513	-4.428	4	0.451	-7.355	-5.586	**0.011**
Ecuador	ECM_1	Difenoconazole	0.528	-1.514	2.571	1	0.901	-1.240	2.297	**1.442**
Ecuador	ECM_1	Epoxiconazole	-0.805	-2.847	1.237	1	0.901	-2.574	0.964	**0.572**
Ecuador	ECM_1	Propiconazole	-0.372	-2.415	1.670	1	0.901	-2.141	1.397	**0.773**
Ecuador	ECQ_10	Difenoconazole	-4.473	-6.515	-2.430	2	0.637	-5.723	-3.222	**0.045**
Ecuador	ECQ_10	Epoxiconazole	-4.464	-6.506	-2.421	2	0.637	-5.714	-3.213	**0.045**
Ecuador	ECQ_10	Propiconazole	-3.046	-5.089	-1.004	2	0.637	-4.297	-1.796	**0.121**
Ecuador	ECQ_20	Difenoconazole	-5.572	-7.615	-3.530	2	0.637	-6.823	-4.322	**0.021**
Ecuador	ECQ_20	Epoxiconazole	-5.624	-7.666	-3.581	2	0.637	-6.874	-4.373	**0.020**
Ecuador	ECQ_20	Propiconazole	-4.753	-6.795	-2.710	2	0.637	-6.003	-3.502	**0.037**
Ecuador	ECU_18	Difenoconazole	-3.258	-5.300	-1.215	1	0.901	-5.027	-1.489	**0.105**
Ecuador	ECU_18	Epoxiconazole	-3.842	-5.884	-1.799	1	0.901	-5.610	-2.073	**0.070**

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Ecuador	ECU_18	Propiconazole	-2.211	-4.253	-0.168	1	0.901	-3.979	-0.442	**0.216**
Ecuador	ECU_2	Difenoconazole	-2.318	-4.360	-0.276	2	0.637	-3.569	-1.067	**0.201**
Ecuador	ECU_2	Epoxiconazole	-3.238	-5.280	-1.195	2	0.637	-4.488	-1.987	**0.106**
Ecuador	ECU_2	Propiconazole	-1.784	-3.826	0.259	2	0.637	-3.034	-0.533	**0.290**
Ecuador	EN_12	Difenoconazole	-5.773	-7.816	-3.731	1	0.901	-7.542	-4.005	**0.018**
Ecuador	EN_12	Epoxiconazole	-6.091	-8.134	-4.049	1	0.901	-7.860	-4.323	**0.015**
Ecuador	EN_12	Propiconazole	-5.193	-7.235	-3.150	1	0.901	-6.962	-3.424	**0.027**
Ecuador	EN_2	Difenoconazole	-7.012	-9.055	-4.970	2	0.637	-8.263	-5.762	**0.008**
Ecuador	EN_2	Epoxiconazole	-6.420	-8.462	-4.377	2	0.637	-7.671	-5.169	**0.012**
Ecuador	EN_2	Propiconazole	-5.695	-7.737	-3.652	2	0.637	-6.945	-4.444	**0.019**
Ecuador	ENB_52	Difenoconazole	-7.302	-9.344	-5.259	2	0.637	-8.552	-6.051	**0.006**
Ecuador	ENB_52	Epoxiconazole	-6.964	-9.006	-4.921	2	0.637	-8.215	-5.713	**0.008**
Ecuador	ENB_52	Propiconazole	-6.283	-8.325	-4.241	3	0.520	-7.304	-5.262	**0.013**
Ecuador	ENB_6	Difenoconazole	-5.503	-7.546	-3.461	1	0.901	-7.272	-3.734	**0.022**
Ecuador	ENB_6	Epoxiconazole	-5.915	-7.957	-3.872	1	0.901	-7.683	-4.146	**0.017**
Ecuador	ENB_6	Propiconazole	-4.623	-6.665	-2.580	1	0.901	-6.391	-2.854	**0.041**
Ecuador	ENB_7	Difenoconazole	-3.890	-5.933	-1.848	1	0.901	-5.659	-2.122	**0.067**
Ecuador	ENB_7	Epoxiconazole	-3.963	-6.005	-1.921	1	0.901	-5.732	-2.194	**0.064**
Ecuador	ENB_7	Propiconazole	-4.075	-6.118	-2.033	1	0.901	-5.844	-2.307	**0.059**
Ecuador	ENP_8	Difenoconazole	-6.269	-8.311	-4.226	1	0.901	-8.037	-4.500	**0.013**
Ecuador	ENP_8	Epoxiconazole	-5.505	-7.547	-3.462	1	0.901	-7.273	-3.736	**0.022**
Ecuador	ENP_8	Propiconazole	-4.712	-6.754	-2.670	1	0.901	-6.481	-2.943	**0.038**
Ecuador	ENR_4	Difenoconazole	-7.240	-9.283	-5.198	1	0.901	-9.009	-5.471	**0.007**
Ecuador	ENR_4	Epoxiconazole	-5.865	-7.907	-3.823	1	0.901	-7.634	-4.096	**0.017**
Ecuador	ENR_4	Propiconazole	-5.714	-7.756	-3.672	1	0.901	-7.483	-3.945	**0.019**
Ecuador	ENV_5	Difenoconazole	-7.481	-9.523	-5.438	1	0.901	-9.249	-5.712	**0.006**
Ecuador	ENV_5	Epoxiconazole	-7.223	-9.265	-5.180	1	0.901	-8.992	-5.454	**0.007**
Ecuador	ENV_5	Propiconazole	-6.054	-8.096	-4.011	1	0.901	-7.823	-4.285	**0.015**
Ecuador	ENV_9	Difenoconazole	-7.966	-10.008	-5.923	1	0.901	-9.734	-6.197	**<0.004**
Ecuador	ENV_9	Epoxiconazole	-7.332	-9.375	-5.290	3	0.520	-8.354	-6.311	**0.006**
Ecuador	ENV_9	Propiconazole	-6.701	-8.743	-4.658	3	0.520	-7.722	-5.680	**0.010**
Ecuador	ESM_2	Difenoconazole	-5.556	-7.599	-3.514	2	0.637	-6.807	-4.306	**0.021**
Ecuador	ESM_2	Epoxiconazole	-5.125	-7.167	-3.082	2	0.637	-6.375	-3.874	**0.029**
Ecuador	ESM_2	Propiconazole	-4.539	-6.581	-2.496	2	0.637	-5.789	-3.288	**0.043**
Ecuador	ESM_3	Difenoconazole	-5.867	-7.910	-3.825	2	0.637	-7.118	-4.617	**0.017**
Ecuador	ESM_3	Epoxiconazole	-5.596	-7.638	-3.553	2	0.637	-6.847	-4.345	**0.021**
Ecuador	ESM_3	Propiconazole	-5.257	-7.300	-3.215	2	0.637	-6.508	-4.007	**0.026**
Ecuador	ESM_4	Difenoconazole	-4.121	-6.163	-2.078	1	0.901	-5.889	-2.352	**0.057**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Ecuador	ESM_4	Epoxiconazole	-4.240	-6.282	-2.198	1	0.901	-6.009	-2.471	**0.053**
Ecuador	ESM_4	Propiconazole	-4.205	-6.247	-2.162	1	0.901	-5.973	-2.436	**0.054**
Colombia	Esmeraldas_1	Difenoconazole	2.596	0.553	4.638	3	0.520	1.575	3.617	**6.046**
Colombia	Esmeraldas_1	Epoxiconazole	1.475	-0.567	3.517	3	0.520	0.454	2.496	**2.780**
Colombia	Esmeraldas_1	Propiconazole	2.080	0.037	4.122	3	0.520	1.058	3.101	**4.227**
Colombia	Esmeraldas_2	Difenoconazole	2.896	0.853	4.938	1	0.901	1.127	4.664	**7.442**
Colombia	Esmeraldas_2	Epoxiconazole	1.028	-1.014	3.070	1	0.901	-0.741	2.797	**2.039**
Colombia	Esmeraldas_2	Propiconazole	1.725	-0.317	3.768	1	0.901	-0.043	3.494	**3.307**
Colombia	Esmeraldas_3	Difenoconazole	1.650	-0.392	3.692	3	0.520	0.629	2.671	**3.139**
Colombia	Esmeraldas_3	Epoxiconazole	1.103	-0.939	3.145	3	0.520	0.082	2.124	**2.148**
Colombia	Esmeraldas_3	Propiconazole	1.067	-0.975	3.110	3	0.520	0.046	2.088	**2.095**
Colombia	Esmeraldas_4	Difenoconazole	0.626	-1.416	2.669	1	0.901	-1.142	2.395	**1.544**
Colombia	Esmeraldas_4	Epoxiconazole	-0.231	-2.274	1.811	1	0.901	-2.000	1.537	**0.852**
Colombia	Esmeraldas_4	Propiconazole	1.048	-0.995	3.090	1	0.901	-0.721	2.816	**2.067**
Colombia	Esmeraldas_5	Difenoconazole	2.355	0.313	4.398	1	0.901	0.586	4.124	**5.116**
Colombia	Esmeraldas_5	Epoxiconazole	0.556	-1.486	2.598	1	0.901	-1.213	2.325	**1.470**
Colombia	Esmeraldas_5	Propiconazole	1.542	-0.500	3.585	1	0.901	-0.226	3.311	**2.913**
Colombia	Esperanza_4	Difenoconazole	2.331	0.288	4.373	1	0.901	0.562	4.099	**5.031**
Colombia	Esperanza_4	Epoxiconazole	2.067	0.025	4.110	1	0.901	0.299	3.836	**4.191**
Colombia	Esperanza_4	Propiconazole	3.047	1.004	5.089	1	0.901	1.278	4.815	**8.263**
Ecuador	ESS_2	Difenoconazole	-6.654	-8.696	-4.611	2	0.637	-7.904	-5.403	**0.010**
Ecuador	ESS_2	Epoxiconazole	-6.077	-8.119	-4.034	2	0.637	-7.327	-4.826	**0.015**
Ecuador	ESS_2	Propiconazole	-5.952	-7.994	-3.909	2	0.637	-7.202	-4.701	**0.016**
Ecuador	ESS_4	Difenoconazole	-3.932	-5.975	-1.890	1	0.901	-5.701	-2.164	**0.065**
Ecuador	ESS_4	Epoxiconazole	-4.594	-6.636	-2.551	1	0.901	-6.362	-2.825	**0.041**
Ecuador	ESS_4	Propiconazole	-5.460	-7.503	-3.418	1	0.901	-7.229	-3.692	**0.023**
Ecuador	ESS_6	Difenoconazole	-6.794	-8.837	-4.752	1	0.901	-8.563	-5.026	**0.009**
Ecuador	ESS_6	Epoxiconazole	-5.512	-7.554	-3.470	1	0.901	-7.281	-3.743	**0.022**
Ecuador	ESS_6	Propiconazole	-6.337	-8.380	-4.295	1	0.901	-8.106	-4.569	**0.012**
Ecuador	ESS_7	Difenoconazole	-7.459	-9.501	-5.416	1	0.901	-9.228	-5.690	**0.006**
Ecuador	ESS_7	Epoxiconazole	-6.274	-8.316	-4.231	1	0.901	-8.043	-4.505	**0.013**
Ecuador	ESS_7	Propiconazole	-6.466	-8.509	-4.424	1	0.901	-8.235	-4.697	**0.011**
Colombia	Estadero_1	Difenoconazole				0				**>10.24**
Colombia	Estadero_1	Epoxiconazole	2.392	0.349	4.434	1	0.901	0.623	4.160	**5.248**
Colombia	Estadero_1	Propiconazole				0				**>10.24**
Colombia	Estadero_2	Difenoconazole	1.380	-0.663	3.422	3	0.520	0.359	2.401	**2.602**
Colombia	Estadero_2	Epoxiconazole	0.025	-2.017	2.068	3	0.520	-0.996	1.047	**1.018**
Colombia	Estadero_2	Propiconazole	0.110	-1.932	2.152	3	0.520	-0.911	1.131	**1.079**

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Colombia	Estadero_3	Difenoconazole	1.469	-0.573	3.512	3	0.520	0.448	2.490	**2.769**
Colombia	Estadero_3	Epoxiconazole	-0.903	-2.945	1.139	3	0.520	-1.924	0.118	**0.535**
Colombia	Estadero_3	Propiconazole	1.155	-0.887	3.197	3	0.520	0.134	2.176	**2.227**
Colombia	Estadero_4	Difenoconazole	-0.090	-2.132	1.953	3	0.520	-1.111	0.931	**0.940**
Colombia	Estadero_4	Epoxiconazole	-0.763	-2.805	1.280	3	0.520	-1.784	0.259	**0.589**
Colombia	Estadero_4	Propiconazole	-0.025	-2.068	2.017	3	0.520	-1.047	0.996	**0.983**
Colombia	Estadero_5	Difenoconazole	2.144	0.101	4.186	3	0.520	1.123	3.165	**4.419**
Colombia	Estadero_5	Epoxiconazole	-0.424	-2.466	1.619	3	0.520	-1.445	0.597	**0.745**
Colombia	Estadero_5	Propiconazole	2.208	0.166	4.251	3	0.520	1.187	3.229	**4.621**
Colombia	Frontera_2	Difenoconazole	0.955	-1.087	2.998	3	0.520	-0.066	1.976	**1.939**
Colombia	Frontera_2	Epoxiconazole	-0.659	-2.701	1.383	3	0.520	-1.680	0.362	**0.633**
Colombia	Frontera_2	Propiconazole	1.230	-0.812	3.273	3	0.520	0.209	2.252	**2.346**
Colombia	Frontera_3	Difenoconazole	0.512	-1.530	2.555	3	0.520	-0.509	1.533	**1.426**
Colombia	Frontera_3	Epoxiconazole	-0.642	-2.685	1.400	3	0.520	-1.663	0.379	**0.641**
Colombia	Frontera_3	Propiconazole	0.278	-1.764	2.320	3	0.520	-0.743	1.299	**1.213**
Colombia	Frontera_5	Difenoconazole	0.747	-1.296	2.789	1	0.901	-1.022	2.515	**1.678**
Colombia	Frontera_5	Epoxiconazole	0.152	-1.891	2.194	2	0.637	-1.099	1.402	**1.111**
Colombia	Frontera_5	Propiconazole	1.967	-0.075	4.009	3	0.520	0.946	2.988	**3.910**
Colombia	Frontera_6	Difenoconazole	2.499	0.456	4.541	1	0.901	0.730	4.268	**5.652**
Colombia	Frontera_6	Epoxiconazole	-0.075	-2.117	1.968	1	0.901	-1.844	1.694	**0.949**
Colombia	Frontera_6	Propiconazole	2.444	0.401	4.486	1	0.901	0.675	4.212	**5.440**
Colombia	Frontera_7	Difenoconazole	2.316	0.274	4.358	1	0.901	0.547	4.085	**4.979**
Colombia	Frontera_7	Epoxiconazole	2.358	0.315	4.400	1	0.901	0.589	4.126	**5.125**
Colombia	Frontera_7	Propiconazole	2.724	0.682	4.767	1	0.901	0.955	4.493	**6.608**
Colombia	Galvis_1	Difenoconazole	-0.852	-2.894	1.191	1	0.901	-2.620	0.917	**0.554**
Colombia	Galvis_1	Epoxiconazole	-0.768	-2.810	1.275	1	0.901	-2.537	1.001	**0.587**
Colombia	Galvis_1	Propiconazole	1.397	-0.645	3.439	1	0.901	-0.372	3.166	**2.633**
Colombia	Galvis_2	Difenoconazole	-1.426	-3.468	0.617	3	0.520	-2.447	-0.404	**0.372**
Colombia	Galvis_2	Epoxiconazole	-2.364	-4.406	-0.321	3	0.520	-3.385	-1.342	**0.194**
Colombia	Galvis_2	Propiconazole	-0.099	-2.141	1.944	3	0.520	-1.120	0.922	**0.934**
Ecuador	GCB_28	Difenoconazole	-1.058	-3.100	0.984	2	0.637	-2.309	0.193	**0.480**
Ecuador	GCB_28	Epoxiconazole	-2.424	-4.467	-0.382	2	0.637	-3.675	-1.173	**0.186**
Ecuador	GCB_28	Propiconazole	-1.146	-3.188	0.897	2	0.637	-2.397	0.105	**0.452**
Ecuador	GCB_30	Difenoconazole	-1.424	-3.467	0.618	2	0.637	-2.675	-0.173	**0.373**
Ecuador	GCB_30	Epoxiconazole	-2.457	-4.499	-0.414	2	0.637	-3.708	-1.206	**0.182**
Ecuador	GCB_30	Propiconazole	-2.207	-4.249	-0.165	2	0.637	-3.458	-0.956	**0.217**
Ecuador	GCB_7	Difenoconazole	-2.888	-4.930	-0.845	2	0.637	-4.138	-1.637	**0.135**
Ecuador	GCB_7	Epoxiconazole	-3.861	-5.903	-1.819	2	0.637	-5.112	-2.610	**0.069**

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Ecuador	GCB_7	Propiconazole	-2.827	-4.870	-0.785	2	0.637	-4.078	-1.576	**0.141**
Ecuador	GCLg_18	Difenoconazole	-1.025	-3.068	1.017	2	0.637	-2.276	0.225	**0.491**
Ecuador	GCLg_18	Epoxiconazole	-2.233	-4.275	-0.190	2	0.637	-3.483	-0.982	**0.213**
Ecuador	GCLg_18	Propiconazole	-1.202	-3.245	0.840	2	0.637	-2.453	0.048	**0.435**
Ecuador	GCMA_4	Difenoconazole	-4.023	-6.065	-1.980	4	0.451	-4.907	-3.138	**0.062**
Ecuador	GCMA_4	Epoxiconazole	-3.845	-5.887	-1.802	4	0.451	-4.729	-2.960	**0.070**
Ecuador	GCMA_4	Propiconazole	-3.277	-5.320	-1.235	4	0.451	-4.162	-2.393	**0.103**
Ecuador	GCMS_7	Difenoconazole	-3.138	-5.181	-1.096	3	0.520	-4.160	-2.117	**0.114**
Ecuador	GCMS_7	Epoxiconazole	-3.859	-5.901	-1.816	3	0.520	-4.880	-2.838	**0.069**
Ecuador	GCMS_7	Propiconazole	-2.636	-4.679	-0.594	3	0.520	-3.658	-1.615	**0.161**
Ecuador	GCSB_13	Difenoconazole	-2.480	-4.522	-0.438	3	0.520	-3.501	-1.459	**0.179**
Ecuador	GCSB_13	Epoxiconazole	-3.076	-5.119	-1.034	3	0.520	-4.097	-2.055	**0.119**
Ecuador	GCSB_13	Propiconazole	-1.882	-3.924	0.161	3	0.520	-2.903	-0.861	**0.271**
Ecuador	GNA_1	Difenoconazole	-0.349	-2.391	1.694	2	0.637	-1.599	0.902	**0.785**
Ecuador	GNA_1	Epoxiconazole	-1.905	-3.947	0.138	2	0.637	-3.155	-0.654	**0.267**
Ecuador	GNA_1	Propiconazole	0.340	-1.702	2.383	2	0.637	-0.910	1.591	**1.266**
Ecuador	GNA_6	Difenoconazole	0.110	-1.932	2.153	2	0.637	-1.141	1.361	**1.079**
Ecuador	GNA_6	Epoxiconazole	-2.038	-4.080	0.004	2	0.637	-3.289	-0.787	**0.243**
Ecuador	GNA_6	Propiconazole	-1.388	-3.431	0.654	2	0.637	-2.639	-0.138	**0.382**
Ecuador	GND_18	Difenoconazole	0.460	-1.582	2.503	3	0.520	-0.561	1.482	**1.376**
Ecuador	GND_18	Epoxiconazole	0.179	-1.864	2.221	3	0.520	-0.843	1.200	**1.132**
Ecuador	GND_18	Propiconazole	1.497	-0.545	3.540	3	0.520	0.476	2.519	**2.823**
Ecuador	GNM_1	Difenoconazole	0.637	-1.406	2.679	1	0.901	-1.132	2.406	**1.555**
Ecuador	GNM_1	Epoxiconazole	-0.992	-3.035	1.050	1	0.901	-2.761	0.776	**0.503**
Ecuador	GNM_1	Propiconazole	-1.204	-3.246	0.839	1	0.901	-2.973	0.565	**0.434**
Ecuador	GNMe_1	Difenoconazole	-1.155	-3.197	0.888	1	0.901	-2.923	0.614	**0.449**
Ecuador	GNMe_1	Epoxiconazole	-0.849	-2.891	1.194	1	0.901	-2.618	0.920	**0.555**
Ecuador	GNMe_1	Propiconazole	-1.607	-3.650	0.435	1	0.901	-3.376	0.161	**0.328**
Ecuador	GNP_3	Difenoconazole	1.438	-0.604	3.480	5	0.403	0.647	2.229	**2.709**
Ecuador	GNP_3	Epoxiconazole	0.668	-1.375	2.710	5	0.403	-0.123	1.459	**1.589**
Ecuador	GNP_3	Propiconazole	2.420	0.377	4.462	4	0.451	1.535	3.304	**5.351**
Ecuador	GSa_10	Difenoconazole	-2.520	-4.562	-0.477	2	0.637	-3.771	-1.269	**0.174**
Ecuador	GSa_10	Epoxiconazole	-3.154	-5.196	-1.111	2	0.637	-4.404	-1.903	**0.112**
Ecuador	GSa_10	Propiconazole	-2.429	-4.471	-0.386	2	0.637	-3.679	-1.178	**0.186**
Ecuador	GSa_13	Difenoconazole	-2.283	-4.326	-0.241	3	0.520	-3.305	-1.262	**0.205**
Ecuador	GSa_13	Epoxiconazole	-3.177	-5.219	-1.134	3	0.520	-4.198	-2.155	**0.111**
Ecuador	GSa_13	Propiconazole	-1.786	-3.828	0.257	3	0.520	-2.807	-0.765	**0.290**
Ecuador	GSa_2	Difenoconazole	-2.639	-4.682	-0.597	2	0.637	-3.890	-1.389	**0.160**

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Ecuador	GSa_2	Epoxiconazole	-2.945	-4.987	-0.902	2	0.637	-4.196	-1.694	**0.130**
Ecuador	GSa_2	Propiconazole	-1.458	-3.500	0.584	2	0.637	-2.709	-0.207	**0.364**
Ecuador	GSa_4	Difenoconazole	-3.568	-5.610	-1.526	1	0.901	-5.337	-1.799	**0.084**
Ecuador	GSa_4	Epoxiconazole	-4.515	-6.557	-2.473	1	0.901	-6.284	-2.746	**0.044**
Ecuador	GSa_4	Propiconazole	-2.394	-4.437	-0.352	1	0.901	-4.163	-0.626	**0.190**
Ecuador	GSaN_12	Difenoconazole	-2.752	-4.794	-0.709	2	0.637	-4.003	-1.501	**0.148**
Ecuador	GSaN_12	Epoxiconazole	-3.485	-5.528	-1.443	2	0.637	-4.736	-2.234	**0.089**
Ecuador	GSaN_12	Propiconazole	-2.233	-4.276	-0.191	2	0.637	-3.484	-0.983	**0.213**
Ecuador	GSaN_83	Difenoconazole				0				**<0.004**
Ecuador	GSaN_83	Epoxiconazole				0				**<0.004**
Ecuador	GSaN_83	Propiconazole				0				**<0.004**
Ecuador	GSB_11	Difenoconazole	-4.317	-6.359	-2.274	3	0.520	-5.338	-3.296	**0.050**
Ecuador	GSB_11	Epoxiconazole	-4.309	-6.351	-2.266	3	0.520	-5.330	-3.287	**0.050**
Ecuador	GSB_11	Propiconazole	-2.382	-4.425	-0.340	3	0.520	-3.403	-1.361	**0.192**
Ecuador	GSB_5	Difenoconazole	-2.109	-4.152	-0.067	3	0.520	-3.130	-1.088	**0.232**
Ecuador	GSB_5	Epoxiconazole	-2.795	-4.837	-0.752	3	0.520	-3.816	-1.773	**0.144**
Ecuador	GSB_5	Propiconazole	-1.939	-3.981	0.103	3	0.520	-2.960	-0.918	**0.261**
Ecuador	GSB_7	Difenoconazole	-0.308	-2.351	1.734	4	0.451	-1.193	0.576	**0.808**
Ecuador	GSB_7	Epoxiconazole	-1.276	-3.318	0.767	4	0.451	-2.160	-0.391	**0.413**
Ecuador	GSB_7	Propiconazole	0.008	-2.034	2.051	4	0.451	-0.876	0.893	**1.006**
Ecuador	GSB_9	Difenoconazole	-2.388	-4.430	-0.345	1	0.901	-4.157	-0.619	**0.191**
Ecuador	GSB_9	Epoxiconazole	-3.401	-5.444	-1.359	1	0.901	-5.170	-1.632	**0.095**
Ecuador	GSB_9	Propiconazole	-1.823	-3.866	0.219	1	0.901	-3.592	-0.054	**0.283**
Ecuador	GSN_1	Difenoconazole	-1.368	-3.411	0.674	3	0.520	-2.389	-0.347	**0.387**
Ecuador	GSN_1	Epoxiconazole	-2.376	-4.419	-0.334	3	0.520	-3.398	-1.355	**0.193**
Ecuador	GSN_1	Propiconazole	-1.504	-3.547	0.538	3	0.520	-2.525	-0.483	**0.353**
Ecuador	GSN_19	Difenoconazole	-3.582	-5.625	-1.540	1	0.901	-5.351	-1.813	**0.083**
Ecuador	GSN_19	Epoxiconazole	-4.012	-6.055	-1.970	1	0.901	-5.781	-2.244	**0.062**
Ecuador	GSN_19	Propiconazole	-2.069	-4.111	-0.027	1	0.901	-3.838	-0.300	**0.238**
Colombia	Guata_1	Difenoconazole	0.568	-1.474	2.611	2	0.637	-0.682	1.819	**1.483**
Colombia	Guata_1	Epoxiconazole	-1.201	-3.244	0.841	2	0.637	-2.452	0.049	**0.435**
Colombia	Guata_1	Propiconazole	-0.092	-2.134	1.951	2	0.637	-1.342	1.159	**0.938**
Guadalupe	GW_1	Difenoconazole	-7.843	-9.885	-5.801	1	0.901	-9.612	-6.074	**0.004**
Guadalupe	GW_1	Epoxiconazole	-7.688	-9.730	-5.645	1	0.901	-9.456	-5.919	**0.005**
Guadalupe	GW_1	Propiconazole	-4.551	-6.593	-2.508	1	0.901	-6.320	-2.782	**0.043**
Guadalupe	GW_10	Difenoconazole	-6.297	-8.339	-4.254	1	0.901	-8.065	-4.528	**0.013**
Guadalupe	GW_10	Epoxiconazole	-5.262	-7.305	-3.220	1	0.901	-7.031	-3.494	**0.026**
Guadalupe	GW_10	Propiconazole	-4.729	-6.772	-2.687	1	0.901	-6.498	-2.960	**0.038**

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Guadalupe	GW_11	Difenoconazole	-7.367	-9.409	-5.325	1	0.901	-9.136	-5.598	**0.006**
Guadalupe	GW_11	Epoxiconazole	-5.965	-8.007	-3.923	1	0.901	-7.734	-4.196	**0.016**
Guadalupe	GW_11	Propiconazole	-5.523	-7.565	-3.480	1	0.901	-7.291	-3.754	**0.022**
Guadalupe	GW_12	Difenoconazole	-5.574	-7.616	-3.531	1	0.901	-7.343	-3.805	**0.021**
Guadalupe	GW_12	Epoxiconazole	-4.715	-6.758	-2.673	1	0.901	-6.484	-2.947	**0.038**
Guadalupe	GW_12	Propiconazole	-4.447	-6.489	-2.405	1	0.901	-6.216	-2.678	**0.046**
Guadalupe	GW_14	Difenoconazole	-7.475	-9.518	-5.433	1	0.901	-9.244	-5.706	**0.006**
Guadalupe	GW_14	Epoxiconazole	-7.176	-9.219	-5.134	1	0.901	-8.945	-5.408	**0.007**
Guadalupe	GW_14	Propiconazole	-6.277	-8.319	-4.234	1	0.901	-8.045	-4.508	**0.013**
Guadalupe	GW_15	Difenoconazole	-7.554	-9.596	-5.511	1	0.901	-9.323	-5.785	**0.005**
Guadalupe	GW_15	Epoxiconazole	-6.347	-8.390	-4.305	1	0.901	-8.116	-4.579	**0.012**
Guadalupe	GW_15	Propiconazole	-6.279	-8.321	-4.237	1	0.901	-8.048	-4.510	**0.013**
Guadalupe	GW_16_8	Difenoconazole	-7.067	-9.109	-5.024	1	0.901	-8.835	-5.298	**0.007**
Guadalupe	GW_16_8	Epoxiconazole	-6.887	-8.930	-4.845	1	0.901	-8.656	-5.119	**0.008**
Guadalupe	GW_16_8	Propiconazole	-6.260	-8.302	-4.217	1	0.901	-8.028	-4.491	**0.013**
Guadalupe	GW_16_9	Difenoconazole	-6.098	-8.141	-4.056	1	0.901	-7.867	-4.330	**0.015**
Guadalupe	GW_16_9	Epoxiconazole	-6.102	-8.145	-4.060	1	0.901	-7.871	-4.333	**0.015**
Guadalupe	GW_16_9	Propiconazole	-5.195	-7.237	-3.152	1	0.901	-6.964	-3.426	**0.027**
Guadalupe	GW_2	Difenoconazole	-7.575	-9.618	-5.533	1	0.901	-9.344	-5.807	**0.005**
Guadalupe	GW_2	Epoxiconazole				0				**<0.004**
Guadalupe	GW_2	Propiconazole	-4.108	-6.151	-2.066	1	0.901	-5.877	-2.340	**0.058**
Guadalupe	GW_26	Difenoconazole	-5.925	-7.968	-3.883	1	0.901	-7.694	-4.156	**0.016**
Guadalupe	GW_26	Epoxiconazole	-5.795	-7.837	-3.752	1	0.901	-7.563	-4.026	**0.018**
Guadalupe	GW_26	Propiconazole	-4.398	-6.440	-2.355	1	0.901	-6.166	-2.629	**0.047**
Guadalupe	GW_28	Difenoconazole	-5.791	-7.834	-3.749	1	0.901	-7.560	-4.023	**0.018**
Guadalupe	GW_28	Epoxiconazole	-6.090	-8.132	-4.048	1	0.901	-7.859	-4.321	**0.015**
Guadalupe	GW_28	Propiconazole	-7.843	-9.885	-5.801	1	0.901	-9.612	-6.074	**0.004**
Guadalupe	GW_29	Difenoconazole	-6.107	-8.149	-4.064	1	0.901	-7.876	-4.338	**0.015**
Guadalupe	GW_29	Epoxiconazole	-6.169	-8.211	-4.126	1	0.901	-7.938	-4.400	**0.014**
Guadalupe	GW_29	Propiconazole	-7.575	-9.618	-5.533	1	0.901	-9.344	-5.807	**0.005**
Guadalupe	GW_3	Difenoconazole	-7.519	-9.562	-5.477	1	0.901	-9.288	-5.751	**0.005**
Guadalupe	GW_3	Epoxiconazole	-6.206	-8.248	-4.163	1	0.901	-7.974	-4.437	**0.014**
Guadalupe	GW_3	Propiconazole	-4.344	-6.387	-2.302	1	0.901	-6.113	-2.575	**0.049**
Guadalupe	GW_30	Difenoconazole	-7.519	-9.561	-5.476	1	0.901	-9.288	-5.750	**0.005**
Guadalupe	GW_30	Epoxiconazole	-6.755	-8.797	-4.712	1	0.901	-8.523	-4.986	**0.009**
Guadalupe	GW_30	Propiconazole	-7.519	-9.562	-5.477	1	0.901	-9.288	-5.751	**0.005**
Guadalupe	GW_31	Difenoconazole	-7.507	-9.549	-5.464	1	0.901	-9.276	-5.738	**0.005**
Guadalupe	GW_31	Epoxiconazole	-6.478	-8.520	-4.435	1	0.901	-8.247	-4.709	**0.011**

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Guadalupe	GW_31	Propiconazole	-6.105	-8.148	-4.063	1	0.901	-7.874	-4.337	**0.015**
Guadalupe	GW_33	Difenoconazole	-7.581	-9.624	-5.539	1	0.901	-9.350	-5.812	**0.005**
Guadalupe	GW_33	Epoxiconazole	-6.537	-8.579	-4.494	1	0.901	-8.306	-4.768	**0.011**
Guadalupe	GW_33	Propiconazole	-5.219	-7.261	-3.177	1	0.901	-6.988	-3.450	**0.027**
Guadalupe	GW_34	Difenoconazole	-5.128	-7.170	-3.085	1	0.901	-6.896	-3.359	**0.029**
Guadalupe	GW_34	Epoxiconazole	-4.150	-6.193	-2.108	1	0.901	-5.919	-2.381	**0.056**
Guadalupe	GW_34	Propiconazole	-5.362	-7.405	-3.320	1	0.901	-7.131	-3.594	**0.024**
Guadalupe	GW_35	Difenoconazole	-6.356	-8.399	-4.314	1	0.901	-8.125	-4.588	**0.012**
Guadalupe	GW_35	Epoxiconazole	-6.407	-8.449	-4.365	1	0.901	-8.176	-4.638	**0.012**
Guadalupe	GW_35	Propiconazole	-5.285	-7.328	-3.243	1	0.901	-7.054	-3.517	**0.026**
Guadalupe	GW_36	Difenoconazole	-5.652	-7.694	-3.610	1	0.901	-7.421	-3.883	**0.020**
Guadalupe	GW_36	Epoxiconazole	-5.835	-7.878	-3.793	1	0.901	-7.604	-4.066	**0.018**
Guadalupe	GW_36	Propiconazole	-6.412	-8.454	-4.369	1	0.901	-8.180	-4.643	**0.012**
Guadalupe	GW_39	Difenoconazole	-6.131	-8.173	-4.088	1	0.901	-7.899	-4.362	**0.014**
Guadalupe	GW_39	Epoxiconazole	-6.216	-8.258	-4.173	1	0.901	-7.984	-4.447	**0.013**
Guadalupe	GW_39	Propiconazole	-6.297	-8.339	-4.254	1	0.901	-8.065	-4.528	**0.013**
Guadalupe	GW_4	Difenoconazole	-6.105	-8.148	-4.063	1	0.901	-7.874	-4.337	**0.015**
Guadalupe	GW_4	Epoxiconazole	-6.219	-8.262	-4.177	1	0.901	-7.988	-4.451	**0.013**
Guadalupe	GW_4	Propiconazole	-5.335	-7.377	-3.292	1	0.901	-7.103	-3.566	**0.025**
Guadalupe	GW_41	Difenoconazole	-5.188	-7.231	-3.146	1	0.901	-6.957	-3.419	**0.027**
Guadalupe	GW_41	Epoxiconazole	-5.440	-7.483	-3.398	1	0.901	-7.209	-3.672	**0.023**
Guadalupe	GW_41	Propiconazole	-7.367	-9.409	-5.325	1	0.901	-9.136	-5.598	**0.006**
Guadalupe	GW_42	Difenoconazole	-4.383	-6.426	-2.341	1	0.901	-6.152	-2.614	**0.048**
Guadalupe	GW_42	Epoxiconazole	-6.083	-8.125	-4.041	1	0.901	-7.852	-4.314	**0.015**
Guadalupe	GW_42	Propiconazole	-5.574	-7.616	-3.531	1	0.901	-7.343	-3.805	**0.021**
Guadalupe	GW_43	Difenoconazole	-5.224	-7.266	-3.181	1	0.901	-6.993	-3.455	**0.027**
Guadalupe	GW_43	Epoxiconazole	-5.074	-7.116	-3.032	1	0.901	-6.843	-3.305	**0.030**
Guadalupe	GW_43	Propiconazole	-7.475	-9.518	-5.433	1	0.901	-9.244	-5.706	**0.006**
Guadalupe	GW_44	Difenoconazole	-5.930	-7.972	-3.887	1	0.901	-7.699	-4.161	**0.016**
Guadalupe	GW_44	Epoxiconazole	-5.755	-7.798	-3.713	1	0.901	-7.524	-3.987	**0.019**
Guadalupe	GW_44	Propiconazole	-7.554	-9.596	-5.511	1	0.901	-9.323	-5.785	**0.005**
Guadalupe	GW_46	Difenoconazole	-4.042	-6.084	-2.000	1	0.901	-5.811	-2.273	**0.061**
Guadalupe	GW_46	Epoxiconazole	-3.598	-5.640	-1.555	1	0.901	-5.367	-1.829	**0.083**
Guadalupe	GW_46	Propiconazole	-7.067	-9.109	-5.024	1	0.901	-8.835	-5.298	**0.007**
Guadalupe	GW_5	Difenoconazole	-5.219	-7.261	-3.177	1	0.901	-6.988	-3.450	**0.027**
Guadalupe	GW_5	Epoxiconazole	-5.808	-7.850	-3.766	1	0.901	-7.577	-4.039	**0.018**
Guadalupe	GW_5	Propiconazole	-5.354	-7.396	-3.311	1	0.901	-7.122	-3.585	**0.024**
Guadalupe	GW_6	Difenoconazole	-5.362	-7.405	-3.320	1	0.901	-7.131	-3.594	**0.024**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Guadalupe	GW_6	Epoxiconazole	-4.458	-6.501	-2.416	1	0.901	-6.227	-2.690	**0.045**
Guadalupe	GW_6	Propiconazole	-5.284	-7.326	-3.241	1	0.901	-7.053	-3.515	**0.026**
Guadalupe	GW_7	Difenoconazole	-5.285	-7.328	-3.243	1	0.901	-7.054	-3.517	**0.026**
Guadalupe	GW_7	Epoxiconazole	-6.257	-8.300	-4.215	1	0.901	-8.026	-4.489	**0.013**
Guadalupe	GW_7	Propiconazole	-5.640	-7.682	-3.598	1	0.901	-7.409	-3.871	**0.020**
Guadalupe	GW_8	Difenoconazole	-6.412	-8.454	-4.369	1	0.901	-8.180	-4.643	**0.012**
Guadalupe	GW_8	Epoxiconazole	-6.216	-8.258	-4.174	1	0.901	-7.985	-4.447	**0.013**
Guadalupe	GW_8	Propiconazole	-5.607	-7.649	-3.565	1	0.901	-7.376	-3.838	**0.021**
Colombia	Horizonte_1	Difenoconazole	-0.207	-2.250	1.835	1	0.901	-1.976	1.561	**0.866**
Colombia	Horizonte_1	Epoxiconazole	-1.262	-3.305	0.780	1	0.901	-3.031	0.506	**0.417**
Colombia	Horizonte_1	Propiconazole	-0.978	-3.020	1.065	1	0.901	-2.747	0.791	**0.508**
Colombia	Horizonte_3	Difenoconazole	2.822	0.780	4.865	1	0.901	1.054	4.591	**7.074**
Colombia	Horizonte_3	Epoxiconazole	1.677	-0.365	3.719	3	0.520	0.656	2.698	**3.198**
Colombia	Horizonte_3	Propiconazole	3.025	0.983	5.067	3	0.520	2.004	4.046	**8.139**
Colombia	Horizonte_4	Difenoconazole	1.691	-0.351	3.733	5	0.403	0.900	2.482	**3.229**
Colombia	Horizonte_4	Epoxiconazole	-0.712	-2.754	1.331	6	0.368	-1.434	0.011	**0.611**
Colombia	Horizonte_4	Propiconazole	1.623	-0.419	3.665	6	0.368	0.901	2.345	**3.080**
Colombia	Llorona_1	Difenoconazole	-7.529	-9.571	-5.487	1	0.901	-9.298	-5.760	**0.005**
Colombia	Llorona_1	Epoxiconazole	-5.891	-7.933	-3.849	1	0.901	-7.660	-4.122	**0.017**
Colombia	Llorona_1	Propiconazole	-5.690	-7.732	-3.648	1	0.901	-7.459	-3.921	**0.019**
Colombia	Llorona_2	Difenoconazole	-7.355	-9.398	-5.313	1	0.901	-9.124	-5.587	**0.006**
Colombia	Llorona_2	Epoxiconazole	-6.152	-8.195	-4.110	1	0.901	-7.921	-4.383	**0.014**
Colombia	Llorona_2	Propiconazole	-5.827	-7.869	-3.785	1	0.901	-7.596	-4.058	**0.018**
Colombia	Luisa Fernanda_1	Difenoconazole	3.310	1.268	5.352	1	0.901	1.541	5.079	**9.917**
Colombia	Luisa Fernanda_1	Epoxiconazole	2.524	0.481	4.566	1	0.901	0.755	4.293	**5.751**
Colombia	Luisa Fernanda_1	Propiconazole	3.070	1.027	5.112	1	0.901	1.301	4.839	**8.396**
Colombia	Luisa Fernanda_10	Difenoconazole	0.624	-1.419	2.666	1	0.901	-1.145	2.393	**1.541**
Colombia	Luisa Fernanda_10	Epoxiconazole	-1.036	-3.079	1.006	1	0.901	-2.805	0.733	**0.488**
Colombia	Luisa Fernanda_10	Propiconazole	1.150	-0.892	3.193	1	0.901	-0.619	2.919	**2.220**
Colombia	Luisa Fernanda_2	Difenoconazole	2.656	0.614	4.699	1	0.901	0.888	4.425	**6.305**
Colombia	Luisa Fernanda_2	Epoxiconazole	0.261	-1.781	2.304	1	0.901	-1.507	2.030	**1.199**
Colombia	Luisa Fernanda_2	Propiconazole	1.594	-0.449	3.636	1	0.901	-0.175	3.362	**3.018**
Colombia	Luisa Fernanda_4	Difenoconazole	2.793	0.751	4.836	1	0.901	1.025	4.562	**6.932**
Colombia	Luisa Fernanda_4	Epoxiconazole	1.032	-1.011	3.074	1	0.901	-0.737	2.800	**2.044**
Colombia	Luisa Fernanda_4	Propiconazole	2.688	0.645	4.730	1	0.901	0.919	4.456	**6.442**
Colombia	Luisa Fernanda_5	Difenoconazole	2.160	0.117	4.202	1	0.901	0.391	3.929	**4.469**
Colombia	Luisa Fernanda_5	Epoxiconazole	0.320	-1.723	2.362	1	0.901	-1.449	2.088	**1.248**
Colombia	Luisa Fernanda_5	Propiconazole	0.996	-1.046	3.038	1	0.901	-0.773	2.765	**1.995**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Philippines	M52_1	Difenoconazole	-1.608	-3.651	0.434	3	0.520	-2.630	-0.587	**0.328**
Philippines	M52_1	Epoxiconazole	-2.494	-4.536	-0.452	3	0.520	-3.515	-1.473	**0.177**
Philippines	M52_1	Propiconazole	-0.366	-2.408	1.676	3	0.520	-1.387	0.655	**0.776**
Philippines	M52_10	Difenoconazole	-1.375	-3.417	0.668	3	0.520	-2.396	-0.354	**0.386**
Philippines	M52_10	Epoxiconazole	-2.045	-4.087	-0.002	3	0.520	-3.066	-1.024	**0.242**
Philippines	M52_10	Propiconazole	0.189	-1.853	2.231	3	0.520	-0.832	1.210	**1.140**
Philippines	M52_11	Difenoconazole	-0.860	-2.903	1.182	1	0.901	-2.629	0.908	**0.551**
Philippines	M52_11	Epoxiconazole	-1.940	-3.982	0.102	1	0.901	-3.709	-0.171	**0.261**
Philippines	M52_11	Propiconazole	1.021	-1.022	3.063	1	0.901	-0.748	2.789	**2.029**
Philippines	M52_12	Difenoconazole	-0.940	-2.982	1.103	1	0.901	-2.709	0.829	**0.521**
Philippines	M52_12	Epoxiconazole	-0.249	-2.292	1.793	1	0.901	-2.018	1.519	**0.841**
Philippines	M52_12	Propiconazole	1.965	-0.077	4.008	1	0.901	0.196	3.734	**3.905**
Philippines	M52_13	Difenoconazole	1.302	-0.741	3.344	1	0.901	-0.467	3.071	**2.465**
Philippines	M52_13	Epoxiconazole	-0.020	-2.063	2.022	1	0.901	-1.789	1.748	**0.986**
Philippines	M52_13	Propiconazole	2.421	0.378	4.463	1	0.901	0.652	4.190	**5.354**
Philippines	M52_14	Difenoconazole	-0.389	-2.431	1.654	3	0.520	-1.410	0.632	**0.764**
Philippines	M52_14	Epoxiconazole	-0.536	-2.579	1.506	3	0.520	-1.557	0.485	**0.690**
Philippines	M52_14	Propiconazole	-0.707	-2.750	1.335	3	0.520	-1.729	0.314	**0.612**
Philippines	M52_15	Difenoconazole	0.965	-1.077	3.007	1	0.901	-0.804	2.734	**1.952**
Philippines	M52_15	Epoxiconazole	1.924	-0.118	3.966	1	0.901	0.155	3.693	**3.795**
Philippines	M52_15	Propiconazole	1.088	-0.954	3.131	1	0.901	-0.680	2.857	**2.126**
Philippines	M52_16	Difenoconazole	1.192	-0.850	3.235	1	0.901	-0.576	2.961	**2.285**
Philippines	M52_16	Epoxiconazole	1.605	-0.437	3.647	1	0.901	-0.164	3.374	**3.042**
Philippines	M52_16	Propiconazole	0.855	-1.187	2.897	1	0.901	-0.914	2.624	**1.809**
Philippines	M52_17	Difenoconazole	-1.720	-3.763	0.322	1	0.901	-3.489	0.048	**0.303**
Philippines	M52_17	Epoxiconazole	-1.059	-3.101	0.984	1	0.901	-2.828	0.710	**0.480**
Philippines	M52_17	Propiconazole	-0.750	-2.792	1.292	1	0.901	-2.519	1.019	**0.595**
Philippines	M52_18	Difenoconazole	-1.716	-3.758	0.327	1	0.901	-3.484	0.053	**0.304**
Philippines	M52_18	Epoxiconazole	-0.885	-2.927	1.157	1	0.901	-2.654	0.884	**0.542**
Philippines	M52_18	Propiconazole	0.295	-1.748	2.337	1	0.901	-1.474	2.064	**1.227**
Philippines	M52_19	Difenoconazole	-2.285	-4.328	-0.243	1	0.901	-4.054	-0.517	**0.205**
Philippines	M52_19	Epoxiconazole	-1.321	-3.363	0.722	1	0.901	-3.089	0.448	**0.400**
Philippines	M52_19	Propiconazole	-0.390	-2.433	1.652	1	0.901	-2.159	1.378	**0.763**
Philippines	M52_2	Difenoconazole	-0.187	-2.230	1.855	1	0.901	-1.956	1.582	**0.878**
Philippines	M52_2	Epoxiconazole	0.526	-1.516	2.568	1	0.901	-1.243	2.295	**1.440**
Philippines	M52_2	Propiconazole	0.374	-1.668	2.416	1	0.901	-1.395	2.143	**1.296**
Philippines	M52_20	Difenoconazole	-1.552	-3.595	0.490	1	0.901	-3.321	0.217	**0.341**
Philippines	M52_20	Epoxiconazole	-0.052	-2.094	1.991	1	0.901	-1.821	1.717	**0.965**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Philippines	M52_20	Propiconazole	-2.591	-4.634	-0.549	1	0.901	-4.360	-0.823	**0.166**
Philippines	M52_21	Difenoconazole	-1.144	-3.187	0.898	1	0.901	-2.913	0.624	**0.452**
Philippines	M52_21	Epoxiconazole	-2.409	-4.451	-0.366	1	0.901	-4.177	-0.640	**0.188**
Philippines	M52_21	Propiconazole	-3.341	-5.384	-1.299	1	0.901	-5.110	-1.573	**0.099**
Philippines	M52_22	Difenoconazole	0.853	-1.190	2.895	4	0.451	-0.032	1.737	**1.806**
Philippines	M52_22	Epoxiconazole	-0.309	-2.351	1.734	4	0.451	-1.193	0.576	**0.807**
Philippines	M52_22	Propiconazole	0.428	-1.614	2.470	4	0.451	-0.456	1.312	**1.345**
Philippines	M52_23	Difenoconazole	-0.972	-3.015	1.070	1	0.901	-2.741	0.797	**0.510**
Philippines	M52_23	Epoxiconazole	-1.187	-3.230	0.855	1	0.901	-2.956	0.581	**0.439**
Philippines	M52_23	Propiconazole	1.488	-0.555	3.530	1	0.901	-0.281	3.256	**2.804**
Philippines	M52_24	Difenoconazole	-2.185	-4.227	-0.142	3	0.520	-3.206	-1.163	**0.220**
Philippines	M52_24	Epoxiconazole	-2.978	-5.020	-0.935	3	0.520	-3.999	-1.957	**0.127**
Philippines	M52_24	Propiconazole	0.297	-1.746	2.339	3	0.520	-0.725	1.318	**1.228**
Philippines	M52_25	Difenoconazole	-3.422	-5.465	-1.380	1	0.901	-5.191	-1.653	**0.093**
Philippines	M52_25	Epoxiconazole	-2.997	-5.039	-0.954	1	0.901	-4.765	-1.228	**0.125**
Philippines	M52_25	Propiconazole	-0.749	-2.792	1.293	1	0.901	-2.518	1.019	**0.595**
Philippines	M52_3	Difenoconazole	-0.147	-2.190	1.895	3	0.520	-1.168	0.874	**0.903**
Philippines	M52_3	Epoxiconazole	-1.199	-3.242	0.843	3	0.520	-2.221	-0.178	**0.435**
Philippines	M52_3	Propiconazole	1.194	-0.849	3.236	3	0.520	0.172	2.215	**2.287**
Philippines	M52_35	Difenoconazole	-1.329	-3.372	0.713	1	0.901	-3.098	0.439	**0.398**
Philippines	M52_35	Epoxiconazole	-1.409	-3.452	0.633	1	0.901	-3.178	0.360	**0.377**
Philippines	M52_35	Propiconazole	-0.418	-2.460	1.624	1	0.901	-2.187	1.351	**0.748**
Philippines	M52_37	Difenoconazole	-0.516	-2.559	1.526	1	0.901	-2.285	1.252	**0.699**
Philippines	M52_37	Epoxiconazole	-1.499	-3.541	0.544	1	0.901	-3.267	0.270	**0.354**
Philippines	M52_37	Propiconazole	1.280	-0.762	3.323	1	0.901	-0.488	3.049	**2.429**
Philippines	M52_4	Difenoconazole	-2.077	-4.119	-0.034	3	0.520	-3.098	-1.055	**0.237**
Philippines	M52_4	Epoxiconazole	-2.745	-4.787	-0.702	3	0.520	-3.766	-1.723	**0.149**
Philippines	M52_4	Propiconazole	-0.703	-2.746	1.339	3	0.520	-1.725	0.318	**0.614**
Philippines	M52_5	Difenoconazole	-3.341	-5.384	-1.299	1	0.901	-5.110	-1.573	**0.099**
Philippines	M52_5	Epoxiconazole	-2.482	-4.525	-0.440	1	0.901	-4.251	-0.714	**0.179**
Philippines	M52_5	Propiconazole	-0.795	-2.837	1.248	1	0.901	-2.564	0.974	**0.576**
Philippines	M52_6	Difenoconazole	-2.575	-4.618	-0.533	1	0.901	-4.344	-0.807	**0.168**
Philippines	M52_6	Epoxiconazole	-2.368	-4.411	-0.326	1	0.901	-4.137	-0.599	**0.194**
Philippines	M52_6	Propiconazole	0.041	-2.001	2.084	1	0.901	-1.727	1.810	**1.029**
Philippines	M52_7	Difenoconazole	1.488	-0.555	3.530	1	0.901	-0.281	3.256	**2.804**
Philippines	M52_7	Epoxiconazole	1.259	-0.783	3.301	1	0.901	-0.510	3.028	**2.393**
Philippines	M52_7	Propiconazole	-0.213	-2.255	1.830	1	0.901	-1.981	1.556	**0.863**
Philippines	M52_8	Difenoconazole	2.918	0.875	4.960	1	0.901	1.149	4.686	**7.556**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Philippines	M52_8	Epoxiconazole	3.036	0.993	5.078	1	0.901	1.267	4.804	**8.200**
Philippines	M52_8	Propiconazole	2.864	0.821	4.906	1	0.901	1.095	4.633	**7.279**
Philippines	M52_9	Difenoconazole	-0.936	-2.978	1.107	3	0.520	-1.957	0.086	**0.523**
Philippines	M52_9	Epoxiconazole	-1.572	-3.615	0.470	3	0.520	-2.593	-0.551	**0.336**
Philippines	M52_9	Propiconazole	-0.578	-2.620	1.465	3	0.520	-1.599	0.443	**0.670**
Martinique	Ma26_10	Difenoconazole	-6.217	-8.260	-4.175	1	0.901	-7.986	-4.449	**0.013**
Martinique	Ma26_10	Epoxiconazole	-5.364	-7.407	-3.322	1	0.901	-7.133	-3.595	**0.024**
Martinique	Ma26_10	Propiconazole	-7.507	-9.549	-5.464	1	0.901	-9.276	-5.738	**0.005**
Martinique	Ma26_11	Difenoconazole	-4.795	-6.837	-2.752	1	0.901	-6.563	-3.026	**0.036**
Martinique	Ma26_11	Epoxiconazole	-4.482	-6.524	-2.439	1	0.901	-6.251	-2.713	**0.045**
Martinique	Ma26_11	Propiconazole	-7.581	-9.624	-5.539	1	0.901	-9.350	-5.812	**0.005**
Martinique	Ma26_13	Difenoconazole	-5.904	-7.946	-3.861	1	0.901	-7.673	-4.135	**0.017**
Martinique	Ma26_13	Epoxiconazole	-4.239	-6.282	-2.197	1	0.901	-6.008	-2.470	**0.053**
Martinique	Ma26_13	Propiconazole	-5.128	-7.170	-3.085	1	0.901	-6.896	-3.359	**0.029**
Martinique	Ma26_14	Difenoconazole	-4.665	-6.707	-2.623	1	0.901	-6.434	-2.896	**0.039**
Martinique	Ma26_14	Epoxiconazole	-4.324	-6.367	-2.282	1	0.901	-6.093	-2.556	**0.050**
Martinique	Ma26_14	Propiconazole	-6.356	-8.399	-4.314	1	0.901	-8.125	-4.588	**0.012**
Martinique	Ma26_16	Difenoconazole	-5.119	-7.162	-3.077	1	0.901	-6.888	-3.350	**0.029**
Martinique	Ma26_16	Epoxiconazole	-5.117	-7.159	-3.075	1	0.901	-6.886	-3.348	**0.029**
Martinique	Ma26_16	Propiconazole	-5.652	-7.694	-3.610	1	0.901	-7.421	-3.883	**0.020**
Martinique	Ma26_17	Difenoconazole	-6.059	-8.101	-4.016	1	0.901	-7.828	-4.290	**0.015**
Martinique	Ma26_17	Epoxiconazole	-5.941	-7.983	-3.898	1	0.901	-7.709	-4.172	**0.016**
Martinique	Ma26_17	Propiconazole	-6.131	-8.173	-4.088	1	0.901	-7.899	-4.362	**0.014**
Martinique	Ma26_18	Difenoconazole	-3.792	-5.834	-1.750	1	0.901	-5.561	-2.023	**0.072**
Martinique	Ma26_18	Epoxiconazole	-4.828	-6.870	-2.785	1	0.901	-6.596	-3.059	**0.035**
Martinique	Ma26_18	Propiconazole	-5.188	-7.231	-3.146	1	0.901	-6.957	-3.419	**0.027**
Martinique	Ma26_19	Difenoconazole	-4.073	-6.115	-2.030	1	0.901	-5.841	-2.304	**0.059**
Martinique	Ma26_19	Epoxiconazole	-4.368	-6.410	-2.326	1	0.901	-6.137	-2.599	**0.048**
Martinique	Ma26_19	Propiconazole	-4.383	-6.426	-2.341	1	0.901	-6.152	-2.614	**0.048**
Martinique	Ma26_2	Difenoconazole	-4.538	-6.580	-2.495	1	0.901	-6.307	-2.769	**0.043**
Martinique	Ma26_2	Epoxiconazole	-5.349	-7.392	-3.307	1	0.901	-7.118	-3.581	**0.025**
Martinique	Ma26_2	Propiconazole	-6.098	-8.141	-4.056	1	0.901	-7.867	-4.330	**0.015**
Martinique	Ma26_20	Difenoconazole	-5.952	-7.994	-3.910	1	0.901	-7.721	-4.183	**0.016**
Martinique	Ma26_20	Epoxiconazole	-4.913	-6.955	-2.870	1	0.901	-6.681	-3.144	**0.033**
Martinique	Ma26_20	Propiconazole	-5.224	-7.266	-3.181	1	0.901	-6.993	-3.455	**0.027**
Martinique	Ma26_22	Difenoconazole	-6.368	-8.410	-4.326	1	0.901	-8.137	-4.599	**0.012**
Martinique	Ma26_22	Epoxiconazole	-4.713	-6.756	-2.671	1	0.901	-6.482	-2.944	**0.038**
Martinique	Ma26_22	Propiconazole	-5.930	-7.972	-3.887	1	0.901	-7.699	-4.161	**0.016**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Martinique	Ma26_23	Difenoconazole	-7.622	-9.664	-5.579	1	0.901	-9.391	-5.853	**0.005**
Martinique	Ma26_23	Epoxiconazole	-5.524	-7.566	-3.481	1	0.901	-7.292	-3.755	**0.022**
Martinique	Ma26_23	Propiconazole	-4.042	-6.084	-2.000	1	0.901	-5.811	-2.273	**0.061**
Martinique	Ma26_24	Difenoconazole	-6.088	-8.130	-4.045	1	0.901	-7.856	-4.319	**0.015**
Martinique	Ma26_24	Epoxiconazole	-4.480	-6.522	-2.438	1	0.901	-6.249	-2.711	**0.045**
Martinique	Ma26_24	Propiconazole	-4.538	-6.580	-2.495	1	0.901	-6.307	-2.769	**0.043**
Martinique	Ma26_25	Difenoconazole	-5.510	-7.552	-3.467	1	0.901	-7.279	-3.741	**0.022**
Martinique	Ma26_25	Epoxiconazole	-3.878	-5.921	-1.836	1	0.901	-5.647	-2.110	**0.068**
Martinique	Ma26_25	Propiconazole	-3.544	-5.587	-1.502	1	0.901	-5.313	-1.775	**0.086**
Martinique	Ma26_26	Difenoconazole	-5.309	-7.351	-3.267	1	0.901	-7.078	-3.540	**0.025**
Martinique	Ma26_26	Epoxiconazole	-5.619	-7.661	-3.576	1	0.901	-7.387	-3.850	**0.020**
Martinique	Ma26_26	Propiconazole	-6.273	-8.316	-4.231	1	0.901	-8.042	-4.505	**0.013**
Martinique	Ma26_27	Difenoconazole	-4.498	-6.540	-2.455	1	0.901	-6.267	-2.729	**0.044**
Martinique	Ma26_27	Epoxiconazole	-4.282	-6.324	-2.240	1	0.901	-6.051	-2.513	**0.051**
Martinique	Ma26_27	Propiconazole				0				**<0.004**
Martinique	Ma26_28	Difenoconazole	-4.061	-6.104	-2.019	1	0.901	-5.830	-2.293	**0.060**
Martinique	Ma26_28	Epoxiconazole	-4.760	-6.803	-2.718	1	0.901	-6.529	-2.992	**0.037**
Martinique	Ma26_28	Propiconazole	-4.918	-6.961	-2.876	1	0.901	-6.687	-3.150	**0.033**
Martinique	Ma26_29	Difenoconazole	-7.167	-9.209	-5.124	1	0.901	-8.936	-5.398	**0.007**
Martinique	Ma26_29	Epoxiconazole	-6.262	-8.305	-4.220	1	0.901	-8.031	-4.493	**0.013**
Martinique	Ma26_29	Propiconazole	-6.217	-8.260	-4.175	1	0.901	-7.986	-4.449	**0.013**
Martinique	Ma26_3	Difenoconazole	-3.544	-5.587	-1.502	1	0.901	-5.313	-1.775	**0.086**
Martinique	Ma26_3	Epoxiconazole	-3.572	-5.614	-1.529	1	0.901	-5.340	-1.803	**0.084**
Martinique	Ma26_3	Propiconazole	-5.925	-7.968	-3.883	1	0.901	-7.694	-4.156	**0.016**
Martinique	Ma26_30	Difenoconazole	-7.535	-9.577	-5.492	1	0.901	-9.304	-5.766	**0.005**
Martinique	Ma26_30	Epoxiconazole	-7.014	-9.057	-4.972	1	0.901	-8.783	-5.246	**0.008**
Martinique	Ma26_30	Propiconazole	-6.888	-8.930	-4.845	1	0.901	-8.657	-5.119	**0.008**
Martinique	Ma26_32	Difenoconazole	-7.695	-9.737	-5.653	1	0.901	-9.464	-5.926	**0.005**
Martinique	Ma26_32	Epoxiconazole	-7.626	-9.668	-5.583	1	0.901	-9.394	-5.857	**0.005**
Martinique	Ma26_32	Propiconazole	-6.320	-8.362	-4.277	1	0.901	-8.088	-4.551	**0.013**
Martinique	Ma26_33	Difenoconazole	-6.134	-8.177	-4.092	1	0.901	-7.903	-4.366	**0.014**
Martinique	Ma26_33	Epoxiconazole	-3.337	-5.380	-1.295	1	0.901	-5.106	-1.568	**0.099**
Martinique	Ma26_33	Propiconazole	-5.193	-7.235	-3.151	1	0.901	-6.962	-3.424	**0.027**
Martinique	Ma26_35	Difenoconazole	-6.077	-8.120	-4.035	1	0.901	-7.846	-4.308	**0.015**
Martinique	Ma26_35	Epoxiconazole	-4.648	-6.691	-2.606	1	0.901	-6.417	-2.880	**0.040**
Martinique	Ma26_35	Propiconazole	-6.640	-8.682	-4.597	1	0.901	-8.409	-4.871	**0.010**
Martinique	Ma26_36	Difenoconazole	-4.120	-6.163	-2.078	1	0.901	-5.889	-2.352	**0.057**
Martinique	Ma26_36	Epoxiconazole	-4.333	-6.376	-2.291	1	0.901	-6.102	-2.565	**0.050**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Martinique	Ma26_36	Propiconazole	-4.521	-6.564	-2.479	1	0.901	-6.290	-2.752	**0.044**
Martinique	Ma26_37	Difenoconazole	-6.129	-8.172	-4.087	1	0.901	-7.898	-4.360	**0.014**
Martinique	Ma26_37	Epoxiconazole	-5.831	-7.873	-3.788	1	0.901	-7.600	-4.062	**0.018**
Martinique	Ma26_37	Propiconazole	-5.406	-7.449	-3.364	1	0.901	-7.175	-3.637	**0.024**
Martinique	Ma26_40	Difenoconazole	-7.507	-9.549	-5.465	1	0.901	-9.276	-5.738	**0.005**
Martinique	Ma26_40	Epoxiconazole	-7.264	-9.307	-5.222	1	0.901	-9.033	-5.496	**0.007**
Martinique	Ma26_40	Propiconazole	-6.197	-8.240	-4.155	1	0.901	-7.966	-4.428	**0.014**
Martinique	Ma26_5	Difenoconazole	-6.273	-8.316	-4.231	1	0.901	-8.042	-4.505	**0.013**
Martinique	Ma26_5	Epoxiconazole	-6.122	-8.165	-4.080	1	0.901	-7.891	-4.354	**0.014**
Martinique	Ma26_5	Propiconazole	-5.791	-7.834	-3.749	1	0.901	-7.560	-4.023	**0.018**
Martinique	Ma26_7	Difenoconazole				0				**<0.004**
Martinique	Ma26_7	Epoxiconazole				0				**<0.004**
Martinique	Ma26_7	Propiconazole	-6.107	-8.149	-4.064	1	0.901	-7.876	-4.338	**0.015**
Martinique	Ma26_9	Difenoconazole	-4.918	-6.961	-2.876	1	0.901	-6.687	-3.150	**0.033**
Martinique	Ma26_9	Epoxiconazole	-4.460	-6.502	-2.417	1	0.901	-6.228	-2.691	**0.045**
Martinique	Ma26_9	Propiconazole	-7.519	-9.561	-5.476	1	0.901	-9.288	-5.750	**0.005**
Martinique	Ma27_1	Difenoconazole	-7.695	-9.737	-5.652	1	0.901	-9.464	-5.926	**0.005**
Martinique	Ma27_1	Epoxiconazole	-6.193	-8.235	-4.150	1	0.901	-7.962	-4.424	**0.014**
Martinique	Ma27_1	Propiconazole	-6.314	-8.356	-4.272	1	0.901	-8.083	-4.545	**0.013**
Martinique	Ma27_11	Difenoconazole	-7.594	-9.636	-5.551	1	0.901	-9.362	-5.825	**0.005**
Martinique	Ma27_11	Epoxiconazole	-6.857	-8.899	-4.814	1	0.901	-8.626	-5.088	**0.009**
Martinique	Ma27_11	Propiconazole	-6.070	-8.113	-4.028	1	0.901	-7.839	-4.301	**0.015**
Martinique	Ma27_12	Difenoconazole	-7.602	-9.645	-5.560	1	0.901	-9.371	-5.834	**0.005**
Martinique	Ma27_12	Epoxiconazole	-6.785	-8.827	-4.742	1	0.901	-8.553	-5.016	**0.009**
Martinique	Ma27_12	Propiconazole	-6.624	-8.666	-4.581	1	0.901	-8.392	-4.855	**0.010**
Martinique	Ma27_13	Difenoconazole	-5.488	-7.530	-3.445	1	0.901	-7.257	-3.719	**0.022**
Martinique	Ma27_13	Epoxiconazole	-5.134	-7.177	-3.092	1	0.901	-6.903	-3.365	**0.028**
Martinique	Ma27_13	Propiconazole	-5.471	-7.513	-3.429	1	0.901	-7.240	-3.702	**0.023**
Martinique	Ma27_14	Difenoconazole	-6.011	-8.053	-3.968	1	0.901	-7.780	-4.242	**0.016**
Martinique	Ma27_14	Epoxiconazole	-5.749	-7.792	-3.707	1	0.901	-7.518	-3.981	**0.019**
Martinique	Ma27_14	Propiconazole	-5.589	-7.632	-3.547	1	0.901	-7.358	-3.821	**0.021**
Martinique	Ma27_16	Difenoconazole	-7.286	-9.328	-5.244	1	0.901	-9.055	-5.517	**0.006**
Martinique	Ma27_16	Epoxiconazole	-6.910	-8.952	-4.868	1	0.901	-8.679	-5.141	**0.008**
Martinique	Ma27_16	Propiconazole	-6.484	-8.527	-4.442	1	0.901	-8.253	-4.716	**0.011**
Martinique	Ma27_17	Difenoconazole	-7.813	-9.856	-5.771	1	0.901	-9.582	-6.044	**0.004**
Martinique	Ma27_17	Epoxiconazole	-7.459	-9.501	-5.416	1	0.901	-9.227	-5.690	**0.006**
Martinique	Ma27_17	Propiconazole	-6.124	-8.166	-4.081	1	0.901	-7.893	-4.355	**0.014**
Martinique	Ma27_19	Difenoconazole	-7.733	-9.775	-5.690	1	0.901	-9.501	-5.964	**0.005**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Martinique	Ma27_19	Epoxiconazole	-7.207	-9.250	-5.165	1	0.901	-8.976	-5.438	**0.007**
Martinique	Ma27_19	Propiconazole	-6.192	-8.234	-4.149	1	0.901	-7.960	-4.423	**0.014**
Martinique	Ma27_22	Difenoconazole				0				**<0.004**
Martinique	Ma27_22	Epoxiconazole	-7.821	-9.864	-5.779	1	0.901	-9.590	-6.053	**0.004**
Martinique	Ma27_22	Propiconazole	-7.775	-9.817	-5.733	1	0.901	-9.544	-6.006	**0.005**
Martinique	Ma27_5	Difenoconazole	-6.055	-8.097	-4.012	1	0.901	-7.824	-4.286	**0.015**
Martinique	Ma27_5	Epoxiconazole	-6.189	-8.231	-4.146	1	0.901	-7.958	-4.420	**0.014**
Martinique	Ma27_5	Propiconazole	-4.731	-6.773	-2.688	1	0.901	-6.499	-2.962	**0.038**
Martinique	Ma27_6	Difenoconazole	-7.795	-9.837	-5.753	1	0.901	-9.564	-6.026	**0.005**
Martinique	Ma27_6	Epoxiconazole	-7.510	-9.553	-5.468	2	0.637	-8.761	-6.260	**0.005**
Martinique	Ma27_6	Propiconazole	-6.764	-8.806	-4.722	2	0.637	-8.015	-5.513	**0.009**
Martinique	Ma27_7	Difenoconazole	-5.848	-7.890	-3.805	1	0.901	-7.616	-4.079	**0.017**
Martinique	Ma27_7	Epoxiconazole	-5.484	-7.526	-3.441	1	0.901	-7.253	-3.715	**0.022**
Martinique	Ma27_7	Propiconazole	-5.933	-7.976	-3.891	1	0.901	-7.702	-4.165	**0.016**
Martinique	Ma27_9	Difenoconazole	-5.000	-7.042	-2.957	1	0.901	-6.769	-3.231	**0.031**
Martinique	Ma27_9	Epoxiconazole	-4.451	-6.493	-2.408	1	0.901	-6.220	-2.682	**0.046**
Martinique	Ma27_9	Propiconazole	-4.642	-6.684	-2.600	1	0.901	-6.411	-2.873	**0.040**
Colombia	Montecristo_3	Difenoconazole	2.065	0.023	4.108	1	0.901	0.296	3.834	**4.185**
Colombia	Montecristo_3	Epoxiconazole	-0.109	-2.151	1.934	1	0.901	-1.877	1.660	**0.927**
Colombia	Montecristo_3	Propiconazole	1.152	-0.890	3.195	1	0.901	-0.616	2.921	**2.223**
Colombia	Montecristo_4	Difenoconazole	2.295	0.253	4.338	3	0.520	1.274	3.317	**4.909**
Colombia	Montecristo_4	Epoxiconazole	0.779	-1.263	2.822	3	0.520	-0.242	1.801	**1.716**
Colombia	Montecristo_4	Propiconazole	1.843	-0.199	3.886	3	0.520	0.822	2.865	**3.589**
Colombia	Montecristo_5	Difenoconazole	2.396	0.353	4.438	3	0.520	1.374	3.417	**5.262**
Colombia	Montecristo_5	Epoxiconazole	1.260	-0.783	3.302	3	0.520	0.239	2.281	**2.394**
Colombia	Montecristo_5	Propiconazole	2.194	0.151	4.236	3	0.520	1.172	3.215	**4.575**
Colombia	Montecristo_7	Difenoconazole	2.949	0.907	4.992	1	0.901	1.181	4.718	**7.724**
Colombia	Montecristo_7	Epoxiconazole	0.963	-1.080	3.005	1	0.901	-0.806	2.731	**1.949**
Colombia	Montecristo_7	Propiconazole	1.452	-0.590	3.495	1	0.901	-0.316	3.221	**2.737**
Dominican Rep.	O_1	Difenoconazole	1.518	-0.525	3.560	3	0.520	0.496	2.539	**2.863**
Dominican Rep.	O_1	Epoxiconazole	0.651	-1.392	2.693	3	0.520	-0.371	1.672	**1.570**
Dominican Rep.	O_1	Propiconazole	1.587	-0.455	3.629	3	0.520	0.566	2.608	**3.004**
Dominican Rep.	O_2	Difenoconazole	0.497	-1.546	2.539	3	0.520	-0.524	1.518	**1.411**
Dominican Rep.	O_2	Epoxiconazole	-0.347	-2.390	1.695	3	0.520	-1.369	0.674	**0.786**
Dominican Rep.	O_2	Propiconazole	0.427	-1.616	2.469	3	0.520	-0.594	1.448	**1.344**
Dominican Rep.	O_3	Difenoconazole	0.694	-1.349	2.736	3	0.520	-0.328	1.715	**1.617**
Dominican Rep.	O_3	Epoxiconazole	0.365	-1.677	2.408	3	0.520	-0.656	1.387	**1.288**
Dominican Rep.	O_3	Propiconazole	1.732	-0.311	3.774	3	0.520	0.710	2.753	**3.321**

Country	Isolate	Fungicide	[2]Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Dominican Rep.	O_4	Difenoconazole	1.125	-0.918	3.167	3	0.520	0.104	2.146	**2.181**
Dominican Rep.	O_4	Epoxiconazole	0.577	-1.465	2.620	3	0.520	-0.444	1.599	**1.492**
Dominican Rep.	O_4	Propiconazole	0.702	-1.341	2.744	3	0.520	-0.320	1.723	**1.626**
Dominican Rep.	O_5	Difenoconazole	-0.790	-2.832	1.253	3	0.520	-1.811	0.231	**0.578**
Dominican Rep.	O_5	Epoxiconazole	-0.819	-2.861	1.223	3	0.520	-1.840	0.202	**0.567**
Dominican Rep.	O_5	Propiconazole	-0.168	-2.211	1.874	3	0.520	-1.189	0.853	**0.890**
Ecuador	OCM_11	Difenoconazole	-2.367	-4.409	-0.325	4	0.451	-3.251	-1.483	**0.194**
Ecuador	OCM_11	Epoxiconazole	-3.530	-5.572	-1.487	4	0.451	-4.414	-2.645	**0.087**
Ecuador	OCM_11	Propiconazole	-1.846	-3.889	0.196	4	0.451	-2.731	-0.962	**0.278**
Ecuador	OCM_12	Difenoconazole	-0.234	-2.277	1.808	4	0.451	-1.119	0.650	**0.850**
Ecuador	OCM_12	Epoxiconazole	-1.808	-3.850	0.234	4	0.451	-2.692	-0.924	**0.286**
Ecuador	OCM_12	Propiconazole	0.458	-1.584	2.501	4	0.451	-0.426	1.343	**1.374**
Ecuador	OCM_15	Difenoconazole	0.361	-1.681	2.403	1	0.901	-1.408	2.130	**1.284**
Ecuador	OCM_15	Epoxiconazole	-0.463	-2.505	1.580	1	0.901	-2.231	1.306	**0.726**
Ecuador	OCM_15	Propiconazole	0.519	-1.523	2.562	1	0.901	-1.249	2.288	**1.433**
Ecuador	OCM_20	Difenoconazole	-1.296	-3.339	0.746	1	0.901	-3.065	0.473	**0.407**
Ecuador	OCM_20	Epoxiconazole	-1.342	-3.384	0.700	1	0.901	-3.111	0.427	**0.394**
Ecuador	OCM_20	Propiconazole	-0.181	-2.223	1.862	1	0.901	-1.949	1.588	**0.882**
Ecuador	OCM_26	Difenoconazole	-1.820	-3.862	0.222	1	0.901	-3.589	-0.051	**0.283**
Ecuador	OCM_26	Epoxiconazole	-1.489	-3.532	0.553	1	0.901	-3.258	0.280	**0.356**
Ecuador	OCM_26	Propiconazole	-0.367	-2.409	1.675	1	0.901	-2.136	1.402	**0.775**
Ecuador	OCM_6	Difenoconazole	-1.373	-3.415	0.670	3	0.520	-2.394	-0.351	**0.386**
Ecuador	OCM_6	Epoxiconazole	-2.894	-4.936	-0.852	3	0.520	-3.915	-1.873	**0.135**
Ecuador	OCM_6	Propiconazole	-1.489	-3.531	0.554	3	0.520	-2.510	-0.467	**0.356**
Ecuador	ONM_20	Difenoconazole	0.602	-1.440	2.645	1	0.901	-1.166	2.371	**1.518**
Ecuador	ONM_20	Epoxiconazole	-0.829	-2.872	1.213	1	0.901	-2.598	0.939	**0.563**
Ecuador	ONM_20	Propiconazole	-0.665	-2.708	1.377	1	0.901	-2.434	1.104	**0.631**
Ecuador	ONM_9	Difenoconazole	-0.660	-2.702	1.383	1	0.901	-2.428	1.109	**0.633**
Ecuador	ONM_9	Epoxiconazole	-0.052	-2.095	1.990	1	0.901	-1.821	1.716	**0.964**
Ecuador	ONM_9	Propiconazole	0.029	-2.013	2.072	1	0.901	-1.740	1.798	**1.020**
Ecuador	ONP_2	Difenoconazole	-1.741	-3.783	0.301	1	0.901	-3.510	0.028	**0.299**
Ecuador	ONP_2	Epoxiconazole	-1.959	-4.002	0.083	1	0.901	-3.728	-0.191	**0.257**
Ecuador	ONP_2	Propiconazole	-0.361	-2.404	1.681	1	0.901	-2.130	1.408	**0.779**
Ecuador	ONS_34	Difenoconazole	-2.449	-4.491	-0.407	1	0.901	-4.218	-0.680	**0.183**
Ecuador	ONS_34	Epoxiconazole	-2.419	-4.461	-0.376	1	0.901	-4.187	-0.650	**0.187**
Ecuador	ONS_34	Propiconazole	-1.886	-3.929	0.156	1	0.901	-3.655	-0.117	**0.271**
Ecuador	ONS_51	Difenoconazole	-1.737	-3.779	0.306	1	0.901	-3.505	0.032	**0.300**
Ecuador	ONS_51	Epoxiconazole	-1.367	-3.410	0.675	1	0.901	-3.136	0.402	**0.388**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Ecuador	ONS_51	Propiconazole	0.437	-1.606	2.479	1	0.901	-1.332	2.205	**1.353**
Ecuador	ONS_8	Difenoconazole	0.848	-1.195	2.890	1	0.901	-0.921	2.616	**1.799**
Ecuador	ONS_8	Epoxiconazole	-0.467	-2.509	1.576	1	0.901	-2.236	1.302	**0.724**
Ecuador	ONS_8	Propiconazole	1.164	-0.879	3.206	1	0.901	-0.605	2.932	**2.240**
Ecuador	Osa_19	Difenoconazole	0.901	-1.142	2.943	1	0.901	-0.868	2.669	**1.867**
Ecuador	Osa_19	Epoxiconazole	-0.898	-2.940	1.144	1	0.901	-2.667	0.871	**0.537**
Ecuador	Osa_19	Propiconazole	0.925	-1.118	2.967	1	0.901	-0.844	2.694	**1.898**
Ecuador	Osa_20	Difenoconazole	-1.155	-3.198	0.887	1	0.901	-2.924	0.613	**0.449**
Ecuador	Osa_20	Epoxiconazole	-1.111	-3.153	0.931	1	0.901	-2.880	0.658	**0.463**
Ecuador	Osa_20	Propiconazole	0.770	-1.272	2.813	1	0.901	-0.998	2.539	**1.706**
Ecuador	Osa_22	Difenoconazole	-1.973	-4.015	0.070	1	0.901	-3.742	-0.204	**0.255**
Ecuador	Osa_22	Epoxiconazole	-1.708	-3.750	0.335	1	0.901	-3.477	0.061	**0.306**
Ecuador	Osa_22	Propiconazole	0.798	-1.244	2.841	1	0.901	-0.970	2.567	**1.739**
Ecuador	Osa_23	Difenoconazole	-1.606	-3.648	0.437	1	0.901	-3.375	0.163	**0.329**
Ecuador	Osa_23	Epoxiconazole	-1.896	-3.938	0.147	1	0.901	-3.664	-0.127	**0.269**
Ecuador	Osa_23	Propiconazole	-0.314	-2.356	1.729	1	0.901	-2.082	1.455	**0.805**
Ecuador	Osa_25	Difenoconazole	-1.347	-3.390	0.695	1	0.901	-3.116	0.421	**0.393**
Ecuador	Osa_25	Epoxiconazole	-1.002	-3.045	1.040	1	0.901	-2.771	0.766	**0.499**
Ecuador	Osa_25	Propiconazole	0.051	-1.991	2.094	1	0.901	-1.718	1.820	**1.036**
Ecuador	Osa_31	Difenoconazole	-1.931	-3.973	0.111	1	0.901	-3.700	-0.162	**0.262**
Ecuador	Osa_31	Epoxiconazole	-2.716	-4.758	-0.673	1	0.901	-4.484	-0.947	**0.152**
Ecuador	Osa_31	Propiconazole	-0.836	-2.879	1.206	1	0.901	-2.605	0.933	**0.560**
Ecuador	Osa_32	Difenoconazole	-1.047	-3.089	0.996	1	0.901	-2.815	0.722	**0.484**
Ecuador	Osa_32	Epoxiconazole	-1.326	-3.369	0.716	1	0.901	-3.095	0.442	**0.399**
Ecuador	Osa_32	Propiconazole	-1.402	-3.445	0.640	1	0.901	-3.171	0.367	**0.378**
Ecuador	OSSR_13	Difenoconazole	-2.526	-4.568	-0.483	1	0.901	-4.295	-0.757	**0.174**
Ecuador	OSSR_13	Epoxiconazole	-1.745	-3.787	0.297	1	0.901	-3.514	0.024	**0.298**
Ecuador	OSSR_13	Propiconazole	-0.509	-2.552	1.533	1	0.901	-2.278	1.260	**0.703**
Ecuador	OSSR_35	Difenoconazole	-2.000	-4.042	0.043	1	0.901	-3.769	-0.231	**0.250**
Ecuador	OSSR_35	Epoxiconazole	-1.106	-3.148	0.937	1	0.901	-2.874	0.663	**0.465**
Ecuador	OSSR_35	Propiconazole	-0.103	-2.145	1.940	1	0.901	-1.871	1.666	**0.931**
Ecuador	OSSR_36	Difenoconazole	-0.897	-2.939	1.145	3	0.520	-1.918	0.124	**0.537**
Ecuador	OSSR_36	Epoxiconazole	-1.838	-3.881	0.204	3	0.520	-2.859	-0.817	**0.280**
Ecuador	OSSR_36	Propiconazole	-1.118	-3.160	0.925	3	0.520	-2.139	-0.097	**0.461**
Ecuador	OSSR_51	Difenoconazole	-1.632	-3.675	0.410	1	0.901	-3.401	0.136	**0.323**
Ecuador	OSSR_51	Epoxiconazole	-0.887	-2.929	1.156	1	0.901	-2.655	0.882	**0.541**
Ecuador	OSSR_51	Propiconazole	-0.082	-2.124	1.960	1	0.901	-1.851	1.687	**0.945**
Ecuador	OSSR_87	Difenoconazole	-1.882	-3.924	0.160	1	0.901	-3.651	-0.113	**0.271**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Ecuador	OSSR_87	Epoxiconazole	-1.197	-3.240	0.845	1	0.901	-2.966	0.571	**0.436**
Ecuador	OSSR_87	Propiconazole	0.213	-1.829	2.256	1	0.901	-1.555	1.982	**1.159**
Ecuador	OSSR_96	Difenoconazole	0.637	-1.406	2.679	1	0.901	-1.132	2.406	**1.555**
Ecuador	OSSR_96	Epoxiconazole	-0.992	-3.035	1.050	1	0.901	-2.761	0.776	**0.503**
Ecuador	OSSR_96	Propiconazole	-1.204	-3.246	0.839	1	0.901	-2.973	0.565	**0.434**
Cameroon	P0S_14	Difenoconazole	0.761	-1.281	2.803	3	0.520	-0.260	1.782	**1.695**
Cameroon	P0S_14	Epoxiconazole	0.068	-1.975	2.110	3	0.520	-0.954	1.089	**1.048**
Cameroon	P0S_14	Propiconazole	0.844	-1.199	2.886	3	0.520	-0.177	1.865	**1.795**
Cameroon	P0S_16	Difenoconazole	-6.640	-8.682	-4.598	3	0.520	-7.661	-5.619	**0.010**
Cameroon	P0S_16	Epoxiconazole	-6.086	-8.128	-4.043	3	0.520	-7.107	-5.065	**0.015**
Cameroon	P0S_16	Propiconazole	-4.583	-6.626	-2.541	3	0.520	-5.604	-3.562	**0.042**
Cameroon	P0S_18b	Difenoconazole	-0.483	-2.525	1.559	3	0.520	-1.504	0.538	**0.715**
Cameroon	P0S_18b	Epoxiconazole	-0.596	-2.639	1.446	3	0.520	-1.617	0.425	**0.661**
Cameroon	P0S_18b	Propiconazole	0.486	-1.556	2.529	3	0.520	-0.535	1.508	**1.401**
Cameroon	P0S_22a	Difenoconazole				0				**<0.004**
Cameroon	P0S_22a	Epoxiconazole	-7.805	-9.847	-5.762	2	0.637	-9.056	-6.554	**0.004**
Cameroon	P0S_22a	Propiconazole	-7.305	-9.347	-5.262	2	0.637	-8.556	-6.054	**0.006**
Cameroon	P0S_22b	Difenoconazole	-7.699	-9.741	-5.657	2	0.637	-8.950	-6.448	**0.005**
Cameroon	P0S_22b	Epoxiconazole	-7.233	-9.275	-5.191	3	0.520	-8.254	-6.212	**0.007**
Cameroon	P0S_22b	Propiconazole	-6.458	-8.501	-4.416	3	0.520	-7.479	-5.437	**0.011**
Cameroon	P0S_29	Difenoconazole	0.969	-1.073	3.012	3	0.520	-0.052	1.990	**1.958**
Cameroon	P0S_29	Epoxiconazole	-0.456	-2.498	1.587	3	0.520	-1.477	0.565	**0.729**
Cameroon	P0S_29	Propiconazole	0.988	-1.055	3.030	3	0.520	-0.033	2.009	**1.983**
Cameroon	P0S_38	Difenoconazole	-1.112	-3.154	0.930	3	0.520	-2.133	-0.091	**0.463**
Cameroon	P0S_38	Epoxiconazole	-1.373	-3.415	0.670	3	0.520	-2.394	-0.351	**0.386**
Cameroon	P0S_38	Propiconazole	-0.425	-2.468	1.617	3	0.520	-1.447	0.596	**0.745**
Cameroon	P0S_53	Difenoconazole	-3.381	-5.423	-1.338	1	0.901	-5.150	-1.612	**0.096**
Cameroon	P0S_53	Epoxiconazole	-3.211	-5.253	-1.169	1	0.901	-4.980	-1.442	**0.108**
Cameroon	P0S_53	Propiconazole	-1.276	-3.318	0.767	1	0.901	-3.045	0.493	**0.413**
Cameroon	P0S_54	Difenoconazole	-0.684	-2.726	1.358	3	0.520	-1.705	0.337	**0.622**
Cameroon	P0S_54	Epoxiconazole	-1.226	-3.269	0.816	3	0.520	-2.248	-0.205	**0.427**
Cameroon	P0S_54	Propiconazole	-0.081	-2.123	1.962	3	0.520	-1.102	0.940	**0.946**
Cameroon	P0S_58b	Difenoconazole	-2.398	-4.440	-0.355	2	0.637	-3.648	-1.147	**0.190**
Cameroon	P0S_58b	Epoxiconazole	-3.748	-5.791	-1.706	2	0.637	-4.999	-2.498	**0.074**
Cameroon	P0S_58b	Propiconazole	-1.363	-3.406	0.679	2	0.637	-2.614	-0.112	**0.389**
Cameroon	P0S_59a	Difenoconazole	-0.864	-2.906	1.179	3	0.520	-1.885	0.158	**0.550**
Cameroon	P0S_59a	Epoxiconazole	-2.936	-4.978	-0.894	3	0.520	-3.957	-1.915	**0.131**
Cameroon	P0S_59a	Propiconazole	-0.429	-2.471	1.613	3	0.520	-1.450	0.592	**0.743**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Cameroon	P0S_59b	Difenoconazole	-4.828	-6.871	-2.786	3	0.520	-5.850	-3.807	**0.035**
Cameroon	P0S_59b	Epoxiconazole	-4.622	-6.664	-2.579	3	0.520	-5.643	-3.600	**0.041**
Cameroon	P0S_59b	Propiconazole	-2.174	-4.216	-0.131	3	0.520	-3.195	-1.152	**0.222**
Cameroon	P0S_7a	Difenoconazole	-2.327	-4.369	-0.284	3	0.520	-3.348	-1.306	**0.199**
Cameroon	P0S_7a	Epoxiconazole	-2.747	-4.789	-0.705	3	0.520	-3.768	-1.726	**0.149**
Cameroon	P0S_7a	Propiconazole	-1.344	-3.387	0.698	3	0.520	-2.365	-0.323	**0.394**
Cameroon	P0S_72	Difenoconazole	0.939	-1.103	2.981	3	0.520	-0.082	1.960	**1.917**
Cameroon	P0S_72	Epoxiconazole	0.207	-1.835	2.250	3	0.520	-0.814	1.229	**1.155**
Cameroon	P0S_72	Propiconazole	0.387	-1.655	2.430	3	0.520	-0.634	1.408	**1.308**
Cameroon	P0S_76a	Difenoconazole	-2.352	-4.394	-0.309	3	0.520	-3.373	-1.330	**0.196**
Cameroon	P0S_76a	Epoxiconazole	-2.448	-4.490	-0.405	3	0.520	-3.469	-1.427	**0.183**
Cameroon	P0S_76a	Propiconazole	-1.095	-3.137	0.947	3	0.520	-2.116	-0.074	**0.468**
Cameroon	P0S_84a	Difenoconazole	-6.566	-8.608	-4.523	3	0.520	-7.587	-5.545	**0.011**
Cameroon	P0S_84a	Epoxiconazole	-5.884	-7.926	-3.841	3	0.520	-6.905	-4.863	**0.017**
Cameroon	P0S_84a	Propiconazole	-5.681	-7.724	-3.639	3	0.520	-6.703	-4.660	**0.019**
Cameroon	P0S_84b	Difenoconazole	-1.435	-3.477	0.607	3	0.520	-2.456	-0.414	**0.370**
Cameroon	P0S_84b	Epoxiconazole	-1.574	-3.617	0.468	3	0.520	-2.596	-0.553	**0.336**
Cameroon	P0S_84b	Propiconazole	-0.694	-2.737	1.348	3	0.520	-1.715	0.327	**0.618**
Cameroon	P0S_9	Difenoconazole	-3.224	-5.266	-1.181	3	0.520	-4.245	-2.202	**0.107**
Cameroon	P0S_9	Epoxiconazole	-3.225	-5.268	-1.183	3	0.520	-4.246	-2.204	**0.107**
Cameroon	P0S_9	Propiconazole	-1.565	-3.608	0.477	3	0.520	-2.586	-0.544	**0.338**
Cameroon	P0S_91	Difenoconazole	-4.178	-6.221	-2.136	3	0.520	-5.200	-3.157	**0.055**
Cameroon	P0S_91	Epoxiconazole	-4.097	-6.140	-2.055	3	0.520	-5.118	-3.076	**0.058**
Cameroon	P0S_91	Propiconazole	-2.414	-4.456	-0.372	3	0.520	-3.435	-1.393	**0.188**
Cameroon	P2S_14	Difenoconazole	0.436	-1.606	2.478	2	0.637	-0.815	1.687	**1.353**
Cameroon	P2S_14	Epoxiconazole	0.037	-2.005	2.080	3	0.520	-0.984	1.059	**1.026**
Cameroon	P2S_14	Propiconazole	1.231	-0.812	3.273	3	0.520	0.210	2.252	**2.347**
Cameroon	P2S_16	Difenoconazole	-0.989	-3.031	1.054	3	0.520	-2.010	0.033	**0.504**
Cameroon	P2S_16	Epoxiconazole	-1.566	-3.609	0.476	3	0.520	-2.587	-0.545	**0.338**
Cameroon	P2S_16	Propiconazole	-0.487	-2.530	1.555	3	0.520	-1.509	0.534	**0.713**
Cameroon	P2S_19	Difenoconazole	-0.577	-2.619	1.466	3	0.520	-1.598	0.444	**0.670**
Cameroon	P2S_19	Epoxiconazole	-1.118	-3.160	0.924	3	0.520	-2.139	-0.097	**0.461**
Cameroon	P2S_19	Propiconazole	0.408	-1.634	2.451	3	0.520	-0.613	1.430	**1.327**
Cameroon	P2S_20	Difenoconazole	-0.542	-2.584	1.501	3	0.520	-1.563	0.479	**0.687**
Cameroon	P2S_20	Epoxiconazole	-0.119	-2.161	1.924	3	0.520	-1.140	0.903	**0.921**
Cameroon	P2S_20	Propiconazole	0.316	-1.727	2.358	3	0.520	-0.705	1.337	**1.245**
Cameroon	P2S_24	Difenoconazole	-0.368	-2.411	1.674	3	0.520	-1.390	0.653	**0.775**
Cameroon	P2S_24	Epoxiconazole	-1.718	-3.761	0.324	3	0.520	-2.740	-0.697	**0.304**

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Cameroon	P2S_24	Propiconazole	0.593	-1.449	2.636	3	0.520	-0.428	1.614	**1.509**
Cameroon	P2S_25	Difenoconazole	-1.657	-3.699	0.386	3	0.520	-2.678	-0.636	**0.317**
Cameroon	P2S_25	Epoxiconazole	-1.708	-3.751	0.334	3	0.520	-2.730	-0.687	**0.306**
Cameroon	P2S_25	Propiconazole	-0.372	-2.415	1.670	3	0.520	-1.394	0.649	**0.772**
Cameroon	P2S_31	Difenoconazole	-0.650	-2.693	1.392	3	0.520	-1.672	0.371	**0.637**
Cameroon	P2S_31	Epoxiconazole	-1.261	-3.304	0.781	3	0.520	-2.282	-0.240	**0.417**
Cameroon	P2S_31	Propiconazole	0.207	-1.836	2.249	3	0.520	-0.815	1.228	**1.154**
Cameroon	P2S_37	Difenoconazole	-0.856	-2.898	1.187	3	0.520	-1.877	0.166	**0.553**
Cameroon	P2S_37	Epoxiconazole	-1.794	-3.837	0.248	3	0.520	-2.816	-0.773	**0.288**
Cameroon	P2S_37	Propiconazole	-0.020	-2.063	2.022	3	0.520	-1.041	1.001	**0.986**
Cameroon	P2S_40	Difenoconazole	-0.484	-2.527	1.558	3	0.520	-1.506	0.537	**0.715**
Cameroon	P2S_40	Epoxiconazole	-1.025	-3.067	1.018	3	0.520	-2.046	-0.004	**0.491**
Cameroon	P2S_40	Propiconazole	0.056	-1.987	2.098	3	0.520	-0.966	1.077	**1.039**
Cameroon	P2S_41	Difenoconazole	0.163	-1.879	2.206	3	0.520	-0.858	1.185	**1.120**
Cameroon	P2S_41	Epoxiconazole	-0.738	-2.781	1.304	3	0.520	-1.760	0.283	**0.599**
Cameroon	P2S_41	Propiconazole	0.725	-1.318	2.767	3	0.520	-0.296	1.746	**1.653**
Cameroon	P2S_42	Difenoconazole	-0.896	-2.939	1.146	3	0.520	-1.917	0.125	**0.537**
Cameroon	P2S_42	Epoxiconazole	-0.764	-2.806	1.279	3	0.520	-1.785	0.257	**0.589**
Cameroon	P2S_42	Propiconazole	0.267	-1.775	2.310	3	0.520	-0.754	1.289	**1.204**
Cameroon	P2S_44	Difenoconazole	-1.025	-3.068	1.017	3	0.520	-2.047	-0.004	**0.491**
Cameroon	P2S_44	Epoxiconazole	-1.571	-3.613	0.472	3	0.520	-2.592	-0.549	**0.337**
Cameroon	P2S_44	Propiconazole	-0.541	-2.583	1.502	3	0.520	-1.562	0.480	**0.687**
Cameroon	P2S_47	Difenoconazole	0.603	-1.439	2.646	3	0.520	-0.418	1.624	**1.519**
Cameroon	P2S_47	Epoxiconazole	0.244	-1.799	2.286	3	0.520	-0.778	1.265	**1.184**
Cameroon	P2S_47	Propiconazole	0.467	-1.575	2.509	3	0.520	-0.554	1.488	**1.382**
Cameroon	P2S_62	Difenoconazole	-0.733	-2.775	1.310	3	0.520	-1.754	0.288	**0.602**
Cameroon	P2S_62	Epoxiconazole	-1.742	-3.785	0.300	3	0.520	-2.763	-0.721	**0.299**
Cameroon	P2S_62	Propiconazole	-0.474	-2.517	1.568	3	0.520	-1.496	0.547	**0.720**
Cameroon	P2S_64	Difenoconazole	-1.384	-3.427	0.658	3	0.520	-2.405	-0.363	**0.383**
Cameroon	P2S_64	Epoxiconazole	0.437	-1.605	2.480	3	0.520	-0.584	1.459	**1.354**
Cameroon	P2S_64	Propiconazole	0.994	-1.048	3.037	3	0.520	-0.027	2.016	**1.992**
Cameroon	P2S_68	Difenoconazole	-2.009	-4.051	0.033	3	0.520	-3.030	-0.988	**0.248**
Cameroon	P2S_68	Epoxiconazole	-2.366	-4.409	-0.324	3	0.520	-3.388	-1.345	**0.194**
Cameroon	P2S_68	Propiconazole	-0.606	-2.649	1.436	3	0.520	-1.627	0.415	**0.657**
Cameroon	P2S_7	Difenoconazole	-0.704	-2.746	1.339	3	0.520	-1.725	0.318	**0.614**
Cameroon	P2S_7	Epoxiconazole	-0.882	-2.925	1.160	3	0.520	-1.903	0.139	**0.543**
Cameroon	P2S_7	Propiconazole	-0.167	-2.209	1.876	3	0.520	-1.188	0.854	**0.891**
Cameroon	P2S_78	Difenoconazole	0.130	-1.913	2.172	3	0.520	-0.891	1.151	**1.094**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Cameroon	P2S_78	Epoxiconazole	-0.429	-2.472	1.613	3	0.520	-1.450	0.592	**0.743**
Cameroon	P2S_78	Propiconazole	0.219	-1.824	2.261	3	0.520	-0.803	1.240	**1.164**
Cameroon	P2S_79	Difenoconazole	-0.327	-2.369	1.715	3	0.520	-1.348	0.694	**0.797**
Cameroon	P2S_79	Epoxiconazole	-0.672	-2.715	1.370	3	0.520	-1.694	0.349	**0.627**
Cameroon	P2S_79	Propiconazole	0.926	-1.117	2.968	3	0.520	-0.095	1.947	**1.900**
Cameroon	P2S_81	Difenoconazole	-1.620	-3.663	0.422	3	0.520	-2.642	-0.599	**0.325**
Cameroon	P2S_81	Epoxiconazole	-1.752	-3.794	0.291	3	0.520	-2.773	-0.730	**0.297**
Cameroon	P2S_81	Propiconazole	-0.909	-2.952	1.133	3	0.520	-1.931	0.112	**0.532**
Cameroon	P2S_X	Difenoconazole	-0.819	-2.862	1.223	3	0.520	-1.841	0.202	**0.567**
Cameroon	P2S_X	Epoxiconazole	-1.568	-3.611	0.474	3	0.520	-2.590	-0.547	**0.337**
Cameroon	P2S_X	Propiconazole	-0.342	-2.385	1.700	3	0.520	-1.363	0.679	**0.789**
Cameroon	P4S_1	Difenoconazole	1.029	-1.013	3.071	3	0.520	0.008	2.050	**2.041**
Cameroon	P4S_1	Epoxiconazole	0.699	-1.344	2.741	3	0.520	-0.323	1.720	**1.623**
Cameroon	P4S_1	Propiconazole	1.134	-0.909	3.176	3	0.520	0.113	2.155	**2.194**
Cameroon	P4S_13	Difenoconazole	0.190	-1.852	2.233	3	0.520	-0.831	1.212	**1.141**
Cameroon	P4S_13	Epoxiconazole	-0.036	-2.079	2.006	3	0.520	-1.058	0.985	**0.975**
Cameroon	P4S_13	Propiconazole	-0.305	-2.348	1.737	3	0.520	-1.326	0.716	**0.809**
Cameroon	P4S_16	Difenoconazole	-0.100	-2.143	1.942	3	0.520	-1.121	0.921	**0.933**
Cameroon	P4S_16	Epoxiconazole	-0.247	-2.289	1.795	3	0.520	-1.268	0.774	**0.843**
Cameroon	P4S_16	Propiconazole	0.194	-1.848	2.237	3	0.520	-0.827	1.215	**1.144**
Cameroon	P4S_19	Difenoconazole	-0.775	-2.818	1.267	3	0.520	-1.796	0.246	**0.584**
Cameroon	P4S_19	Epoxiconazole	-1.059	-3.101	0.984	3	0.520	-2.080	-0.037	**0.480**
Cameroon	P4S_19	Propiconazole	0.465	-1.578	2.507	3	0.520	-0.557	1.486	**1.380**
Cameroon	P4S_22	Difenoconazole	0.491	-1.551	2.533	3	0.520	-0.530	1.512	**1.405**
Cameroon	P4S_22	Epoxiconazole	0.137	-1.905	2.180	3	0.520	-0.884	1.159	**1.100**
Cameroon	P4S_22	Propiconazole	0.819	-1.224	2.861	3	0.520	-0.202	1.840	**1.764**
Cameroon	P4S_24	Difenoconazole	-0.884	-2.926	1.159	3	0.520	-1.905	0.137	**0.542**
Cameroon	P4S_24	Epoxiconazole	-0.913	-2.956	1.129	3	0.520	-1.934	0.108	**0.531**
Cameroon	P4S_24	Propiconazole	0.586	-1.456	2.628	3	0.520	-0.435	1.607	**1.501**
Cameroon	P4S_28	Difenoconazole	-1.059	-3.101	0.983	3	0.520	-2.080	-0.038	**0.480**
Cameroon	P4S_28	Epoxiconazole	-1.226	-3.269	0.816	3	0.520	-2.248	-0.205	**0.427**
Cameroon	P4S_28	Propiconazole	0.066	-1.977	2.108	3	0.520	-0.955	1.087	**1.047**
Cameroon	P4S_33	Difenoconazole	-0.028	-2.071	2.014	3	0.520	-1.049	0.993	**0.981**
Cameroon	P4S_33	Epoxiconazole	-0.217	-2.259	1.826	3	0.520	-1.238	0.804	**0.860**
Cameroon	P4S_33	Propiconazole	0.363	-1.680	2.405	3	0.520	-0.659	1.384	**1.286**
Cameroon	P4S_38	Difenoconazole	-1.139	-3.181	0.904	3	0.520	-2.160	-0.118	**0.454**
Cameroon	P4S_38	Epoxiconazole	-1.534	-3.577	0.508	3	0.520	-2.556	-0.513	**0.345**
Cameroon	P4S_38	Propiconazole	-0.884	-2.926	1.159	3	0.520	-1.905	0.137	**0.542**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Cameroon	P4S_42	Difenoconazole	-0.864	-2.906	1.179	3	0.520	-1.885	0.157	**0.549**
Cameroon	P4S_42	Epoxiconazole	-1.112	-3.154	0.930	3	0.520	-2.133	-0.091	**0.463**
Cameroon	P4S_42	Propiconazole	0.431	-1.611	2.473	3	0.520	-0.590	1.452	**1.348**
Cameroon	P4S_47	Difenoconazole	-2.116	-4.159	-0.074	3	0.520	-3.138	-1.095	**0.231**
Cameroon	P4S_47	Epoxiconazole	-3.368	-5.411	-1.326	3	0.520	-4.389	-2.347	**0.097**
Cameroon	P4S_47	Propiconazole	-1.386	-3.429	0.656	3	0.520	-2.408	-0.365	**0.382**
Cameroon	P4S_5	Difenoconazole	0.016	-2.026	2.059	3	0.520	-1.005	1.037	**1.011**
Cameroon	P4S_5	Epoxiconazole	0.290	-1.752	2.332	3	0.520	-0.731	1.311	**1.223**
Cameroon	P4S_5	Propiconazole	0.796	-1.246	2.839	3	0.520	-0.225	1.818	**1.737**
Cameroon	P4S_51	Difenoconazole	-1.419	-3.461	0.623	3	0.520	-2.440	-0.398	**0.374**
Cameroon	P4S_51	Epoxiconazole	-1.591	-3.634	0.451	3	0.520	-2.612	-0.570	**0.332**
Cameroon	P4S_51	Propiconazole	0.152	-1.891	2.194	3	0.520	-0.870	1.173	**1.111**
Cameroon	P4S_53	Difenoconazole	-1.521	-3.564	0.521	3	0.520	-2.542	-0.500	**0.348**
Cameroon	P4S_53	Epoxiconazole	-1.313	-3.355	0.730	3	0.520	-2.334	-0.291	**0.403**
Cameroon	P4S_53	Propiconazole	-0.607	-2.650	1.435	3	0.520	-1.628	0.414	**0.656**
Cameroon	P4S_58	Difenoconazole	-0.795	-2.837	1.248	3	0.520	-1.816	0.226	**0.576**
Cameroon	P4S_58	Epoxiconazole	-0.899	-2.941	1.144	3	0.520	-1.920	0.122	**0.536**
Cameroon	P4S_58	Propiconazole	0.179	-1.864	2.221	3	0.520	-0.842	1.200	**1.132**
Cameroon	P4S_60a	Difenoconazole	-1.033	-3.075	1.010	2	0.637	-2.284	0.218	**0.489**
Cameroon	P4S_60a	Epoxiconazole	-1.050	-3.092	0.993	2	0.637	-2.300	0.201	**0.483**
Cameroon	P4S_60a	Propiconazole	0.129	-1.913	2.172	2	0.637	-1.121	1.380	**1.094**
Cameroon	P4S_60b	Difenoconazole	-0.833	-2.875	1.210	3	0.520	-1.854	0.189	**0.562**
Cameroon	P4S_60b	Epoxiconazole	-0.731	-2.773	1.311	3	0.520	-1.752	0.290	**0.603**
Cameroon	P4S_60b	Propiconazole	0.112	-1.930	2.154	3	0.520	-0.909	1.133	**1.081**
Cameroon	P4S_64	Difenoconazole	2.736	0.694	4.778	3	0.520	1.715	3.757	**6.663**
Cameroon	P4S_64	Epoxiconazole	1.965	-0.078	4.007	3	0.520	0.944	2.986	**3.904**
Cameroon	P4S_64	Propiconazole	2.966	0.924	5.009	2	0.637	1.716	4.217	**7.816**
Cameroon	P4S_65	Difenoconazole	0.322	-1.720	2.364	3	0.520	-0.699	1.343	**1.250**
Cameroon	P4S_65	Epoxiconazole	0.375	-1.667	2.418	3	0.520	-0.646	1.397	**1.297**
Cameroon	P4S_65	Propiconazole	0.439	-1.603	2.482	3	0.520	-0.582	1.460	**1.356**
Cameroon	P4S_7a	Difenoconazole	0.251	-1.792	2.293	3	0.520	-0.771	1.272	**1.190**
Cameroon	P4S_7a	Epoxiconazole	-0.705	-2.747	1.338	3	0.520	-1.726	0.316	**0.614**
Cameroon	P4S_7a	Propiconazole	0.675	-1.368	2.717	3	0.520	-0.347	1.696	**1.596**
Cameroon	P4S_7b	Difenoconazole	0.247	-1.795	2.290	3	0.520	-0.774	1.269	**1.187**
Cameroon	P4S_7b	Epoxiconazole	0.077	-1.965	2.120	3	0.520	-0.944	1.098	**1.055**
Cameroon	P4S_7b	Propiconazole	0.859	-1.184	2.901	3	0.520	-0.162	1.880	**1.814**
Cameroon	P4S_72	Difenoconazole	-0.574	-2.617	1.468	3	0.520	-1.595	0.447	**0.672**
Cameroon	P4S_72	Epoxiconazole	-0.942	-2.985	1.100	3	0.520	-1.964	0.079	**0.520**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Cameroon	P4S_72	Propiconazole	0.441	-1.601	2.484	3	0.520	-0.580	1.462	**1.358**
Cameroon	P4S_78	Difenoconazole	0.932	-1.110	2.975	2	0.637	-0.318	2.183	**1.908**
Cameroon	P4S_78	Epoxiconazole	-1.108	-3.150	0.935	1	0.901	-2.877	0.661	**0.464**
Cameroon	P4S_78	Propiconazole	0.794	-1.248	2.836	2	0.637	-0.457	2.045	**1.734**
Cameroon	P4S_81	Difenoconazole	-1.347	-3.390	0.695	1	0.901	-3.116	0.421	**1.264**
Cameroon	P4S_81	Epoxiconazole	-1.831	-3.874	0.211	1	0.901	-3.600	-0.063	**0.706**
Cameroon	P4S_81	Propiconazole	-0.086	-2.129	1.956	1	0.901	-1.855	1.683	**1.778**
Colombia	Paraguay_1	Difenoconazole	2.741	0.699	4.784	1	0.901	0.972	4.510	**6.686**
Colombia	Paraguay_1	Epoxiconazole	-1.272	-3.314	0.771	1	0.901	-3.040	0.497	**0.414**
Colombia	Paraguay_1	Propiconazole	0.200	-1.843	2.242	1	0.901	-1.569	1.968	**1.148**
Colombia	Pinos_1	Difenoconazole	0.385	-1.658	2.427	1	0.901	-1.384	2.154	**1.306**
Colombia	Pinos_1	Epoxiconazole	-1.303	-3.345	0.740	1	0.901	-3.071	0.466	**0.405**
Colombia	Pinos_1	Propiconazole	-0.245	-2.287	1.798	1	0.901	-2.014	1.524	**0.844**
Colombia	Raices_1	Difenoconazole	1.518	-0.524	3.560	2	0.637	0.267	2.769	**2.864**
Colombia	Raices_1	Epoxiconazole	1.164	-0.879	3.206	2	0.637	-0.087	2.414	**2.240**
Colombia	Raices_1	Propiconazole	1.994	-0.049	4.036	3	0.520	0.972	3.015	**3.983**
Colombia	Raices_2	Difenoconazole				0				**>10.24**
Colombia	Raices_2	Epoxiconazole	2.510	0.467	4.552	1	0.901	0.741	4.279	**5.696**
Colombia	Raices_2	Propiconazole	3.286	1.244	5.329	1	0.901	1.518	5.055	**9.757**
Colombia	Raices_4	Difenoconazole	2.701	0.658	4.743	1	0.901	0.932	4.469	**6.501**
Colombia	Raices_4	Epoxiconazole	1.576	-0.467	3.618	1	0.901	-0.193	3.344	**2.981**
Colombia	Raices_4	Propiconazole	1.931	-0.111	3.974	1	0.901	0.162	3.700	**3.814**
Colombia	Raices_5	Difenoconazole	3.091	1.048	5.133	1	0.901	1.322	4.860	**8.520**
Colombia	Raices_5	Epoxiconazole	0.990	-1.052	3.033	1	0.901	-0.778	2.759	**1.987**
Colombia	Raices_5	Propiconazole	2.734	0.692	4.777	1	0.901	0.966	4.503	**6.655**
Colombia	Raices_6	Difenoconazole	2.602	0.559	4.644	1	0.901	0.833	4.371	**6.071**
Colombia	Raices_6	Epoxiconazole	1.509	-0.533	3.551	1	0.901	-0.260	3.278	**2.846**
Colombia	Raices_6	Propiconazole				0				**>10.24**
Ecuador	RCM_14	Difenoconazole	-0.769	-2.811	1.273	1	0.901	-2.538	1.000	**0.587**
Ecuador	RCM_14	Epoxiconazole	0.750	-1.292	2.793	1	0.901	-1.018	2.519	**1.682**
Ecuador	RCM_14	Propiconazole	0.644	-1.398	2.686	1	0.901	-1.125	2.413	**1.563**
Ecuador	RCM_15	Difenoconazole	-2.268	-4.310	-0.225	2	0.637	-3.518	-1.017	**0.208**
Ecuador	RCM_15	Epoxiconazole	-2.270	-4.312	-0.228	2	0.637	-3.521	-1.019	**0.207**
Ecuador	RCM_15	Propiconazole	-1.865	-3.907	0.178	2	0.637	-3.115	-0.614	**0.275**
Ecuador	RCM_16	Difenoconazole	-2.408	-4.450	-0.365	2	0.637	-3.658	-1.157	**0.188**
Ecuador	RCM_16	Epoxiconazole	-4.044	-6.086	-2.001	2	0.637	-5.294	-2.793	**0.061**
Ecuador	RCM_16	Propiconazole	-2.232	-4.275	-0.190	2	0.637	-3.483	-0.981	**0.213**
Ecuador	RCQS_16	Difenoconazole	1.216	-0.826	3.259	3	0.520	0.195	2.238	**2.324**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Ecuador	RCQS_16	Epoxiconazole	-0.086	-2.128	1.957	3	0.520	-1.107	0.936	**0.942**
Ecuador	RCQS_16	Propiconazole	1.465	-0.577	3.508	3	0.520	0.444	2.486	**2.761**
Ecuador	RCQS_19	Difenoconazole	2.365	0.323	4.408	1	0.901	0.596	4.134	**5.152**
Ecuador	RCQS_19	Epoxiconazole	2.169	0.127	4.212	1	0.901	0.401	3.938	**4.498**
Ecuador	RCQS_19	Propiconazole	-0.860	-2.903	1.182	1	0.901	-2.629	0.908	**0.551**
Ecuador	RCQS_3	Difenoconazole	0.380	-1.663	2.422	5	0.403	-0.411	1.171	**1.301**
Ecuador	RCQS_3	Epoxiconazole	-0.119	-2.161	1.924	5	0.403	-0.910	0.672	**0.921**
Ecuador	RCQS_3	Propiconazole	1.557	-0.486	3.599	5	0.403	0.766	2.348	**2.942**
Colombia	Rena_1	Difenoconazole	-0.350	-2.392	1.693	1	0.901	-2.118	1.419	**0.785**
Colombia	Rena_1	Epoxiconazole	0.679	-1.364	2.721	1	0.901	-1.090	2.447	**1.601**
Colombia	Rena_1	Propiconazole	0.813	-1.229	2.856	1	0.901	-0.956	2.582	**1.757**
Ecuador	RNB_13	Difenoconazole	-0.082	-2.125	1.960	1	0.901	-1.851	1.687	**0.945**
Ecuador	RNB_13	Epoxiconazole	2.493	0.451	4.536	1	0.901	0.724	4.262	**5.630**
Ecuador	RNB_13	Propiconazole	0.366	-1.677	2.408	1	0.901	-1.403	2.135	**1.289**
Ecuador	RNB_18	Difenoconazole	0.569	-1.474	2.611	1	0.901	-1.200	2.337	**1.483**
Ecuador	RNB_18	Epoxiconazole	0.302	-1.740	2.345	1	0.901	-1.466	2.071	**1.233**
Ecuador	RNB_18	Propiconazole	-0.193	-2.235	1.850	1	0.901	-1.962	1.576	**0.875**
Ecuador	RNB_19	Difenoconazole	-1.735	-3.778	0.307	1	0.901	-3.504	0.034	**0.300**
Ecuador	RNB_19	Epoxiconazole	-2.605	-4.647	-0.563	1	0.901	-4.374	-0.836	**0.164**
Ecuador	RNB_19	Propiconazole	-1.289	-3.331	0.753	1	0.901	-3.058	0.480	**0.409**
Ecuador	RNVE_10	Difenoconazole	0.680	-1.362	2.723	1	0.901	-1.089	2.449	**1.602**
Ecuador	RNVE_10	Epoxiconazole	1.358	-0.684	3.400	1	0.901	-0.411	3.127	**2.563**
Ecuador	RNVE_10	Propiconazole	2.094	0.052	4.136	1	0.901	0.325	3.863	**4.269**
Ecuador	RNVP_4	Difenoconazole	-0.709	-2.751	1.333	1	0.901	-2.478	1.060	**0.612**
Ecuador	RNVP_4	Epoxiconazole	1.189	-0.853	3.232	1	0.901	-0.579	2.958	**2.280**
Ecuador	RNVP_4	Propiconazole	0.336	-1.707	2.378	1	0.901	-1.433	2.104	**1.262**
Ecuador	RNVP_8	Difenoconazole	1.045	-0.998	3.087	1	0.901	-0.724	2.814	**2.063**
Ecuador	RNVP_8	Epoxiconazole	-0.187	-2.229	1.855	1	0.901	-1.956	1.582	**0.878**
Ecuador	RNVP_8	Propiconazole	-0.016	-2.059	2.026	1	0.901	-1.785	1.753	**0.989**
Ecuador	RSaB_14	Difenoconazole	-7.446	-9.488	-5.403	1	0.901	-9.214	-5.677	**0.006**
Ecuador	RSaB_14	Epoxiconazole				0				**<0.004**
Ecuador	RSaB_14	Propiconazole	-7.835	-9.878	-5.793	1	0.901	-9.604	-6.067	**0.004**
Ecuador	RSaB_36	Difenoconazole	-1.903	-3.945	0.139	1	0.901	-3.672	-0.134	**0.267**
Ecuador	RSaB_36	Epoxiconazole	-2.078	-4.120	-0.035	1	0.901	-3.846	-0.309	**0.237**
Ecuador	RSaB_36	Propiconazole	-2.001	-4.044	0.041	1	0.901	-3.770	-0.232	**0.250**
Ecuador	RSaB_37	Difenoconazole	-1.856	-3.899	0.186	1	0.901	-3.625	-0.088	**0.276**
Ecuador	RSaB_37	Epoxiconazole	-2.226	-4.268	-0.184	1	0.901	-3.995	-0.457	**0.214**
Ecuador	RSaB_37	Propiconazole	-1.026	-3.068	1.017	1	0.901	-2.794	0.743	**0.491**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Ecuador	RSaB_6	Difenoconazole	-4.783	-6.825	-2.740	1	0.901	-6.551	-3.014	**0.036**
Ecuador	RSaB_6	Epoxiconazole	-5.627	-7.670	-3.585	1	0.901	-7.396	-3.859	**0.020**
Ecuador	RSaB_6	Propiconazole	-3.542	-5.584	-1.499	1	0.901	-5.311	-1.773	**0.086**
Ecuador	RSaV_6	Difenoconazole	-6.074	-8.117	-4.032	1	0.901	-7.843	-4.306	**0.015**
Ecuador	RSaV_6	Epoxiconazole	-4.036	-6.078	-1.993	1	0.901	-5.804	-2.267	**0.061**
Ecuador	RSaV_6	Propiconazole	-4.243	-6.286	-2.201	1	0.901	-6.012	-2.475	**0.053**
Ecuador	RSaV_7	Difenoconazole	-2.583	-4.625	-0.540	1	0.901	-4.351	-0.814	**0.167**
Ecuador	RSaV_7	Epoxiconazole	-1.842	-3.884	0.200	1	0.901	-3.611	-0.073	**0.279**
Ecuador	RSaV_7	Propiconazole	-0.489	-2.531	1.553	1	0.901	-2.258	1.280	**0.712**
Ecuador	RSaV_8	Difenoconazole	-7.000	-9.042	-4.957	3	0.520	-8.021	-5.979	**0.008**
Ecuador	RSaV_8	Epoxiconazole	-5.919	-7.961	-3.877	3	0.520	-6.940	-4.898	**0.017**
Ecuador	RSaV_8	Propiconazole	-5.581	-7.623	-3.538	3	0.520	-6.602	-4.559	**0.021**
Ecuador	RSP_1	Difenoconazole	-2.791	-4.833	-0.748	1	0.901	-4.560	-1.022	**0.145**
Ecuador	RSP_1	Epoxiconazole	-3.746	-5.789	-1.704	1	0.901	-5.515	-1.978	**0.075**
Ecuador	RSP_1	Propiconazole	-2.329	-4.371	-0.286	1	0.901	-4.097	-0.560	**0.199**
Ecuador	RSP_11	Difenoconazole	-2.221	-4.263	-0.179	1	0.901	-3.990	-0.452	**0.214**
Ecuador	RSP_11	Epoxiconazole	-3.497	-5.539	-1.454	1	0.901	-5.265	-1.728	**0.089**
Ecuador	RSP_11	Propiconazole	-1.711	-3.754	0.331	1	0.901	-3.480	0.058	**0.305**
Ecuador	RSP_2	Difenoconazole	-2.571	-4.614	-0.529	3	0.520	-3.592	-1.550	**0.168**
Ecuador	RSP_2	Epoxiconazole	-2.872	-4.915	-0.830	3	0.520	-3.893	-1.851	**0.137**
Ecuador	RSP_2	Propiconazole	-1.668	-3.711	0.374	3	0.520	-2.689	-0.647	**0.315**
Ecuador	RSP_3	Difenoconazole	0.387	-1.655	2.429	1	0.901	-1.382	2.156	**1.308**
Ecuador	RSP_3	Epoxiconazole	0.573	-1.469	2.616	1	0.901	-1.196	2.342	**1.488**
Ecuador	RSP_3	Propiconazole	0.914	-1.129	2.956	1	0.901	-0.855	2.682	**1.884**
Ecuador	RSSB_16	Difenoconazole	-1.414	-3.457	0.628	1	0.901	-3.183	0.355	**0.375**
Ecuador	RSSB_16	Epoxiconazole	-0.676	-2.718	1.367	1	0.901	-2.444	1.093	**0.626**
Ecuador	RSSB_16	Propiconazole	0.051	-1.991	2.094	1	0.901	-1.717	1.820	**1.036**
Ecuador	RSSB_22	Difenoconazole	-1.947	-3.989	0.095	3	0.520	-2.968	-0.926	**0.259**
Ecuador	RSSB_22	Epoxiconazole	-2.827	-4.869	-0.785	3	0.520	-3.848	-1.806	**0.141**
Ecuador	RSSB_22	Propiconazole	-0.923	-2.965	1.119	3	0.520	-1.944	0.098	**0.527**
Ecuador	RSSM_6	Difenoconazole	-1.898	-3.940	0.145	3	0.520	-2.919	-0.877	**0.268**
Ecuador	RSSM_6	Epoxiconazole	-2.650	-4.693	-0.608	3	0.520	-3.671	-1.629	**0.159**
Ecuador	RSSM_6	Propiconazole	-0.923	-2.966	1.119	3	0.520	-1.945	0.098	**0.527**
Colombia	Salvis_1	Difenoconazole	0.272	-1.770	2.314	1	0.901	-1.497	2.041	**1.208**
Colombia	Salvis_1	Epoxiconazole	-1.625	-3.667	0.417	1	0.901	-3.394	0.144	**0.324**
Colombia	Salvis_1	Propiconazole	-1.560	-3.603	0.482	1	0.901	-3.329	0.208	**0.339**
Colombia	Santillana_1	Difenoconazole	0.413	-1.629	2.456	1	0.901	-1.355	2.182	**1.332**
Colombia	Santillana_1	Epoxiconazole	-0.297	-2.339	1.746	1	0.901	-2.066	1.472	**0.814**

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Colombia	Santillana_1	Propiconazole	-0.083	-2.125	1.960	1	0.901	-1.851	1.686	**0.944**
Colombia	Santillana_12	Difenoconazole	2.706	0.664	4.749	1	0.901	0.937	4.475	**6.526**
Colombia	Santillana_12	Epoxiconazole	0.718	-1.324	2.761	1	0.901	-1.050	2.487	**1.645**
Colombia	Santillana_12	Propiconazole	0.920	-1.123	2.962	1	0.901	-0.849	2.688	**1.892**
Colombia	Santillana_13	Difenoconazole	3.149	1.106	5.191	3	0.520	2.127	4.170	**8.868**
Colombia	Santillana_13	Epoxiconazole	0.992	-1.050	3.034	3	0.520	-0.029	2.013	**1.989**
Colombia	Santillana_13	Propiconazole	2.429	0.386	4.471	3	0.520	1.408	3.450	**5.384**
Colombia	Santillana_2	Difenoconazole	1.756	-0.286	3.799	3	0.520	0.735	2.777	**3.378**
Colombia	Santillana_2	Epoxiconazole	0.158	-1.885	2.200	3	0.520	-0.863	1.179	**1.116**
Colombia	Santillana_2	Propiconazole	1.001	-1.041	3.044	3	0.520	-0.020	2.023	**2.002**
Colombia	Santillana_3	Difenoconazole	0.725	-1.317	2.767	1	0.901	-1.044	2.494	**1.653**
Colombia	Santillana_3	Epoxiconazole	-1.607	-3.649	0.436	1	0.901	-3.376	0.162	**0.328**
Colombia	Santillana_3	Propiconazole	0.852	-1.190	2.895	1	0.901	-0.916	2.621	**1.805**
Colombia	Santillana_4	Difenoconazole	2.200	0.158	4.243	2	0.637	0.950	3.451	**4.596**
Colombia	Santillana_4	Epoxiconazole	-0.080	-2.122	1.963	2	0.637	-1.330	1.171	**0.946**
Colombia	Santillana_4	Propiconazole	0.541	-1.501	2.584	2	0.637	-0.709	1.792	**1.455**
Colombia	Santillana_5	Difenoconazole				0				**>10.24**
Colombia	Santillana_5	Epoxiconazole	2.999	0.957	5.042	1	0.901	1.231	4.768	**7.996**
Colombia	Santillana_5	Propiconazole				0				**>10.24**
Colombia	Santillana_6	Difenoconazole				0				**>10.24**
Colombia	Santillana_6	Epoxiconazole	2.729	0.687	4.772	1	0.901	0.960	4.498	**6.631**
Colombia	Santillana_6	Propiconazole	2.135	0.093	4.178	1	0.901	0.367	3.904	**4.393**
Colombia	Sierra_1	Difenoconazole	0.945	-1.098	2.987	1	0.901	-0.824	2.713	**1.925**
Colombia	Sierra_1	Epoxiconazole	0.717	-1.325	2.760	1	0.901	-1.052	2.486	**1.644**
Colombia	Sierra_1	Propiconazole	1.078	-0.965	3.120	1	0.901	-0.691	2.847	**2.111**
Costa Rica	SPM2_1	Difenoconazole	1.410	-0.632	3.453	4	0.451	0.526	2.295	**2.658**
Costa Rica	SPM2_1	Epoxiconazole	0.724	-1.319	2.766	4	0.451	-0.161	1.608	**1.651**
Costa Rica	SPM2_1	Propiconazole	1.486	-0.556	3.529	4	0.451	0.602	2.371	**2.801**
Costa Rica	SPM2_11	Difenoconazole	1.746	-0.296	3.789	2	0.637	0.495	2.997	**3.355**
Costa Rica	SPM2_11	Epoxiconazole	0.597	-1.445	2.640	2	0.637	-0.654	1.848	**1.513**
Costa Rica	SPM2_11	Propiconazole	1.575	-0.468	3.617	2	0.637	0.324	2.826	**2.979**
Costa Rica	SPM2_2	Difenoconazole	0.677	-1.366	2.719	4	0.451	-0.208	1.561	**1.599**
Costa Rica	SPM2_2	Epoxiconazole	0.072	-1.970	2.115	4	0.451	-0.812	0.957	**1.051**
Costa Rica	SPM2_2	Propiconazole	0.404	-1.639	2.446	4	0.451	-0.481	1.288	**1.323**
Costa Rica	SPM2_3	Difenoconazole	1.514	-0.529	3.556	4	0.451	0.629	2.398	**2.855**
Costa Rica	SPM2_3	Epoxiconazole	0.587	-1.455	2.630	4	0.451	-0.297	1.472	**1.502**
Costa Rica	SPM2_3	Propiconazole	0.811	-1.231	2.853	4	0.451	-0.073	1.695	**1.755**
Costa Rica	SPM2_4	Difenoconazole	1.834	-0.208	3.877	4	0.451	0.950	2.719	**3.566**

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Costa Rica	SPM2_4	Epoxiconazole	0.524	-1.519	2.566	4	0.451	-0.361	1.408	**1.438**
Costa Rica	SPM2_4	Propiconazole	1.796	-0.246	3.838	4	0.451	0.912	2.680	**3.472**
Costa Rica	SPM2_5	Difenoconazole	2.240	0.197	4.282	2	0.637	0.989	3.490	**4.722**
Costa Rica	SPM2_5	Epoxiconazole	1.938	-0.105	3.980	2	0.637	0.687	3.188	**3.831**
Costa Rica	SPM2_5	Propiconazole	2.229	0.187	4.271	2	0.637	0.978	3.480	**4.688**
Costa Rica	SPM2_6	Difenoconazole	1.506	-0.536	3.548	2	0.637	0.255	2.757	**2.840**
Costa Rica	SPM2_6	Epoxiconazole	1.149	-0.893	3.192	2	0.637	-0.101	2.400	**2.218**
Costa Rica	SPM2_6	Propiconazole	1.402	-0.640	3.444	2	0.637	0.151	2.653	**2.643**
Costa Rica	SPM2_7	Difenoconazole	1.578	-0.464	3.621	2	0.637	0.328	2.829	**2.986**
Costa Rica	SPM2_7	Epoxiconazole	1.332	-0.711	3.374	2	0.637	0.081	2.582	**2.517**
Costa Rica	SPM2_7	Propiconazole	1.091	-0.951	3.133	2	0.637	-0.160	2.342	**2.130**
Costa Rica	SPM2_8	Difenoconazole	1.214	-0.828	3.257	2	0.637	-0.037	2.465	**2.320**
Costa Rica	SPM2_8	Epoxiconazole	-0.011	-2.053	2.032	2	0.637	-1.261	1.240	**0.993**
Costa Rica	SPM2_8	Propiconazole	0.229	-1.814	2.271	2	0.637	-1.022	1.479	**1.172**
Costa Rica	SPM2_9	Difenoconazole	2.267	0.225	4.310	2	0.637	1.016	3.518	**4.814**
Costa Rica	SPM2_9	Epoxiconazole	1.800	-0.242	3.842	2	0.637	0.549	3.051	**3.482**
Costa Rica	SPM2_9	Propiconazole	2.200	0.158	4.243	2	0.637	0.949	3.451	**4.595**
Costa Rica	SPM3_1	Difenoconazole				0				**>10.24**
Costa Rica	SPM3_1	Epoxiconazole	3.013	0.970	5.055	1	0.901	1.244	4.781	**8.071**
Costa Rica	SPM3_1	Propiconazole	2.945	0.903	4.988	1	0.901	1.176	4.714	**7.702**
Costa Rica	SPM3_2	Difenoconazole	2.892	0.849	4.934	1	0.901	1.123	4.660	**7.420**
Costa Rica	SPM3_2	Epoxiconazole	1.508	-0.535	3.550	1	0.901	-0.261	3.277	**2.844**
Costa Rica	SPM3_2	Propiconazole	1.428	-0.614	3.471	1	0.901	-0.340	3.197	**2.691**
Costa Rica	SPM4_1	Difenoconazole	3.331	1.289	5.374	1	0.901	1.563	5.100	**10.066**
Costa Rica	SPM4_1	Epoxiconazole				0				**>10.24**
Costa Rica	SPM4_1	Propiconazole				0				**>10.24**
Costa Rica	SPM4_10	Difenoconazole				0				**>10.24**
Costa Rica	SPM4_10	Epoxiconazole	2.855	0.813	4.898	1	0.901	1.087	4.624	**7.236**
Costa Rica	SPM4_10	Propiconazole				0				**>10.24**
Costa Rica	SPM4_11	Difenoconazole	2.834	0.791	4.876	1	0.901	1.065	4.602	**7.128**
Costa Rica	SPM4_11	Epoxiconazole	2.459	0.416	4.501	1	0.901	0.690	4.228	**5.498**
Costa Rica	SPM4_11	Propiconazole	2.450	0.408	4.493	1	0.901	0.682	4.219	**5.466**
Costa Rica	SPM4_12	Difenoconazole	3.202	1.160	5.244	1	0.901	1.433	4.971	**9.203**
Costa Rica	SPM4_12	Epoxiconazole	2.369	0.326	4.411	1	0.901	0.600	4.137	**5.164**
Costa Rica	SPM4_12	Propiconazole	3.098	1.056	5.141	1	0.901	1.330	4.867	**8.565**
Costa Rica	SPM4_13	Difenoconazole	1.182	-0.860	3.224	1	0.901	-0.587	2.951	**2.269**
Costa Rica	SPM4_13	Epoxiconazole	0.475	-1.567	2.518	1	0.901	-1.293	2.244	**1.390**
Costa Rica	SPM4_13	Propiconazole	2.217	0.175	4.260	1	0.901	0.449	3.986	**4.651**

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Costa Rica	SPM4_14	Difenoconazole	0.855	-1.187	2.898	1	0.901	-0.913	2.624	**1.809**
Costa Rica	SPM4_14	Epoxiconazole	0.475	-1.567	2.518	1	0.901	-1.293	2.244	**1.390**
Costa Rica	SPM4_14	Propiconazole	2.402	0.360	4.445	1	0.901	0.633	4.171	**5.286**
Costa Rica	SPM4_15	Difenoconazole				0				**>10.24**
Costa Rica	SPM4_15	Epoxiconazole	2.885	0.842	4.927	1	0.901	1.116	4.653	**7.385**
Costa Rica	SPM4_15	Propiconazole				0				**>10.24**
Costa Rica	SPM4_2	Difenoconazole	3.064	1.021	5.106	1	0.901	1.295	4.832	**8.361**
Costa Rica	SPM4_2	Epoxiconazole	2.411	0.368	4.453	1	0.901	0.642	4.179	**5.317**
Costa Rica	SPM4_2	Propiconazole	3.045	1.003	5.088	1	0.901	1.277	4.814	**8.255**
Costa Rica	SPM4_3	Difenoconazole				0				**>10.24**
Costa Rica	SPM4_3	Epoxiconazole	2.739	0.696	4.781	1	0.901	0.970	4.507	**6.674**
Costa Rica	SPM4_3	Propiconazole				0				**>10.24**
Costa Rica	SPM4_4	Difenoconazole	2.742	0.700	4.785	1	0.901	0.974	4.511	**6.692**
Costa Rica	SPM4_4	Epoxiconazole	2.574	0.531	4.616	1	0.901	0.805	4.342	**5.953**
Costa Rica	SPM4_4	Propiconazole	2.849	0.807	4.892	1	0.901	1.080	4.618	**7.206**
Costa Rica	SPM4_5	Difenoconazole	1.808	-0.235	3.850	1	0.901	0.039	3.576	**3.501**
Costa Rica	SPM4_5	Epoxiconazole	1.150	-0.893	3.192	1	0.901	-0.619	2.918	**2.218**
Costa Rica	SPM4_5	Propiconazole	1.226	-0.816	3.269	1	0.901	-0.543	2.995	**2.339**
Costa Rica	SPM4_6	Difenoconazole	2.743	0.700	4.785	1	0.901	0.974	4.511	**6.693**
Costa Rica	SPM4_6	Epoxiconazole	2.419	0.377	4.462	1	0.901	0.651	4.188	**5.349**
Costa Rica	SPM4_6	Propiconazole	3.065	1.022	5.107	1	0.901	1.296	4.833	**8.367**
Costa Rica	SPM4_7	Difenoconazole	3.077	1.035	5.120	1	0.901	1.308	4.846	**8.440**
Costa Rica	SPM4_7	Epoxiconazole				0				**>10.24**
Costa Rica	SPM4_7	Propiconazole	2.329	0.286	4.371	1	0.901	0.560	4.097	**5.023**
Costa Rica	SPM4_8	Difenoconazole	3.245	1.203	5.288	1	0.901	1.477	5.014	**9.482**
Costa Rica	SPM4_8	Epoxiconazole	1.289	-0.754	3.331	1	0.901	-0.480	3.057	**2.443**
Costa Rica	SPM4_8	Propiconazole	2.457	0.415	4.500	1	0.901	0.689	4.226	**5.492**
Costa Rica	SPM4_9	Difenoconazole	1.193	-0.850	3.235	1	0.901	-0.576	2.962	**2.286**
Costa Rica	SPM4_9	Epoxiconazole	0.724	-1.318	2.766	1	0.901	-1.045	2.493	**1.652**
Costa Rica	SPM4_9	Propiconazole	0.908	-1.135	2.950	1	0.901	-0.861	2.676	**1.876**
Costa Rica	SPM5_1	Difenoconazole	-0.161	-2.203	1.881	3	0.520	-1.182	0.860	**0.894**
Costa Rica	SPM5_1	Epoxiconazole	-0.742	-2.784	1.301	3	0.520	-1.763	0.280	**0.598**
Costa Rica	SPM5_1	Propiconazole	1.087	-0.956	3.129	3	0.520	0.065	2.108	**2.124**
Costa Rica	SPM5_2	Difenoconazole	2.635	0.593	4.678	1	0.901	0.866	4.404	**6.212**
Costa Rica	SPM5_2	Epoxiconazole	1.166	-0.877	3.208	1	0.901	-0.603	2.935	**2.244**
Costa Rica	SPM5_2	Propiconazole	1.239	-0.804	3.281	1	0.901	-0.530	3.008	**2.360**
Costa Rica	SPM5_3	Difenoconazole				0				**>10.24**
Costa Rica	SPM5_3	Epoxiconazole	1.849	-0.194	3.891	1	0.901	0.080	3.617	**3.601**

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Costa Rica	SPM5_3	Propiconazole				0				**>10.24**
Costa Rica	SPM6_1	Difenoconazole	2.984	0.941	5.026	3	0.520	1.962	4.005	**7.910**
Costa Rica	SPM6_1	Epoxiconazole	2.153	0.111	4.196	3	0.520	1.132	3.174	**4.448**
Costa Rica	SPM6_1	Propiconazole	2.482	0.440	4.525	3	0.520	1.461	3.504	**5.588**
Costa Rica	SPM6_10	Difenoconazole				0				**>10.24**
Costa Rica	SPM6_10	Epoxiconazole	2.537	0.495	4.580	1	0.901	0.768	4.306	**5.804**
Costa Rica	SPM6_10	Propiconazole				0				**>10.24**
Costa Rica	SPM6_11	Difenoconazole				0				**>10.24**
Costa Rica	SPM6_11	Epoxiconazole	1.024	-1.018	3.066	1	0.901	-0.745	2.793	**2.034**
Costa Rica	SPM6_11	Propiconazole	2.740	0.698	4.782	1	0.901	0.971	4.509	**6.681**
Costa Rica	SPM6_12	Difenoconazole				0				**>10.24**
Costa Rica	SPM6_12	Epoxiconazole	3.252	1.209	5.294	1	0.901	1.483	5.020	**9.525**
Costa Rica	SPM6_12	Propiconazole				0				**>10.24**
Costa Rica	SPM6_13	Difenoconazole	2.970	0.928	5.013	1	0.901	1.202	4.739	**7.837**
Costa Rica	SPM6_13	Epoxiconazole	2.664	0.622	4.707	1	0.901	0.895	4.433	**6.339**
Costa Rica	SPM6_13	Propiconazole	0.953	-1.090	2.995	1	0.901	-0.816	2.722	**1.936**
Costa Rica	SPM6_14	Difenoconazole				0				**>10.24**
Costa Rica	SPM6_14	Epoxiconazole	2.745	0.702	4.787	1	0.901	0.976	4.513	**6.702**
Costa Rica	SPM6_14	Propiconazole	2.796	0.753	4.838	1	0.901	1.027	4.564	**6.943**
Costa Rica	SPM6_15	Difenoconazole				0				**>10.24**
Costa Rica	SPM6_15	Epoxiconazole	2.474	0.431	4.516	1	0.901	0.705	4.242	**5.554**
Costa Rica	SPM6_15	Propiconazole	2.809	0.766	4.851	1	0.901	1.040	4.577	**7.006**
Costa Rica	SPM6_2	Difenoconazole	2.634	0.591	4.676	1	0.901	0.865	4.402	**6.206**
Costa Rica	SPM6_2	Epoxiconazole	1.011	-1.032	3.053	1	0.901	-0.758	2.779	**2.015**
Costa Rica	SPM6_2	Propiconazole	2.675	0.632	4.717	1	0.901	0.906	4.443	**6.385**
Costa Rica	SPM6_3	Difenoconazole				0				**>10.24**
Costa Rica	SPM6_3	Epoxiconazole				0				**>10.24**
Costa Rica	SPM6_3	Propiconazole				0				**>10.24**
Costa Rica	SPM6_4	Difenoconazole				0				**>10.24**
Costa Rica	SPM6_4	Epoxiconazole	2.998	0.956	5.041	1	0.901	1.229	4.767	**7.990**
Costa Rica	SPM6_4	Propiconazole				0				**>10.24**
Costa Rica	SPM6_5	Difenoconazole	2.811	0.769	4.854	1	0.901	1.043	4.580	**7.019**
Costa Rica	SPM6_5	Epoxiconazole	1.149	-0.894	3.191	1	0.901	-0.620	2.917	**2.217**
Costa Rica	SPM6_5	Propiconazole	2.733	0.691	4.776	1	0.901	0.965	4.502	**6.650**
Costa Rica	SPM6_6	Difenoconazole	1.703	-0.339	3.745	1	0.901	-0.066	3.472	**3.256**
Costa Rica	SPM6_6	Epoxiconazole	0.454	-1.588	2.497	1	0.901	-1.314	2.223	**1.370**
Costa Rica	SPM6_6	Propiconazole	0.785	-1.257	2.828	1	0.901	-0.984	2.554	**1.723**
Costa Rica	SPM6_7	Difenoconazole				0				**>10.24**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Costa Rica	SPM6_7	Epoxiconazole	1.651	-0.391	3.693	1	0.901	-0.118	3.420	**3.141**
Costa Rica	SPM6_7	Propiconazole	2.879	0.837	4.921	1	0.901	1.110	4.648	**7.356**
Costa Rica	SPM6_8	Difenoconazole				0				**>10.24**
Costa Rica	SPM6_8	Epoxiconazole	3.188	1.146	5.231	1	0.901	1.420	4.957	**9.117**
Costa Rica	SPM6_8	Propiconazole				0				**>10.24**
Costa Rica	SPM6_9	Difenoconazole				0				**>10.24**
Costa Rica	SPM6_9	Epoxiconazole	2.170	0.127	4.212	1	0.901	0.401	3.938	**4.499**
Costa Rica	SPM6_9	Propiconazole	2.764	0.722	4.806	1	0.901	0.995	4.533	**6.793**
Costa Rica	SPM7_1	Difenoconazole	2.066	0.024	4.108	2	0.637	0.815	3.317	**4.187**
Costa Rica	SPM7_1	Epoxiconazole	0.686	-1.356	2.729	3	0.520	-0.335	1.707	**1.609**
Costa Rica	SPM7_1	Propiconazole	2.366	0.324	4.409	3	0.520	1.345	3.387	**5.156**
Costa Rica	SPM7_2	Difenoconazole	2.624	0.582	4.666	1	0.901	0.855	4.393	**6.164**
Costa Rica	SPM7_2	Epoxiconazole	1.273	-0.770	3.315	1	0.901	-0.496	3.041	**2.416**
Costa Rica	SPM7_2	Propiconazole	1.583	-0.460	3.625	1	0.901	-0.186	3.352	**2.996**
Costa Rica	SPM7_3	Difenoconazole	1.389	-0.654	3.431	1	0.901	-0.380	3.158	**2.619**
Costa Rica	SPM7_3	Epoxiconazole	1.182	-0.860	3.225	1	0.901	-0.587	2.951	**2.269**
Costa Rica	SPM7_3	Propiconazole	1.217	-0.825	3.260	1	0.901	-0.551	2.986	**2.325**
Costa Rica	SPM7_4	Difenoconazole	2.670	0.627	4.712	1	0.901	0.901	4.439	**6.363**
Costa Rica	SPM7_4	Epoxiconazole	1.092	-0.951	3.134	1	0.901	-0.677	2.861	**2.132**
Costa Rica	SPM7_4	Propiconazole	2.634	0.592	4.676	1	0.901	0.865	4.403	**6.207**
Colombia	StaI_4	Difenoconazole	2.830	0.788	4.873	1	0.901	1.062	4.599	**7.113**
Colombia	StaI_4	Epoxiconazole	0.794	-1.249	2.836	1	0.901	-0.975	2.562	**1.734**
Colombia	StaI_4	Propiconazole	2.886	0.844	4.928	1	0.901	1.117	4.655	**7.392**
Philippines	T52_1	Difenoconazole	2.467	0.424	4.509	2	0.637	1.216	3.717	**5.528**
Philippines	T52_1	Epoxiconazole	0.887	-1.156	2.929	3	0.520	-0.135	1.908	**1.849**
Philippines	T52_1	Propiconazole	1.179	-0.864	3.221	2	0.637	-0.072	2.430	**2.264**
Philippines	T52_10	Difenoconazole	2.407	0.364	4.449	3	0.520	1.385	3.428	**5.302**
Philippines	T52_10	Epoxiconazole	1.890	-0.152	3.933	4	0.451	1.006	2.775	**3.707**
Philippines	T52_10	Propiconazole	2.225	0.183	4.268	4	0.451	1.341	3.110	**4.676**
Philippines	T52_12	Difenoconazole	3.094	1.052	5.137	1	0.901	1.326	4.863	**8.541**
Philippines	T52_12	Epoxiconazole				0				**>10.24**
Philippines	T52_12	Propiconazole	2.720	0.678	4.763	1	0.901	0.951	4.489	**6.590**
Philippines	T52_13	Difenoconazole	2.154	0.112	4.197	3	0.520	1.133	3.176	**4.452**
Philippines	T52_13	Epoxiconazole	2.138	0.095	4.180	3	0.520	1.117	3.159	**4.401**
Philippines	T52_13	Propiconazole	2.946	0.903	4.988	3	0.520	1.925	3.967	**7.705**
Philippines	T52_14	Difenoconazole				0				**>10.24**
Philippines	T52_14	Epoxiconazole				0				**>10.24**
Philippines	T52_14	Propiconazole				0				**>10.24**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Philippines	T52_15	Difenoconazole	2.590	0.548	4.632	3	0.520	1.569	3.611	**6.021**
Philippines	T52_15	Epoxiconazole	2.857	0.815	4.899	2	0.637	1.606	4.108	**7.245**
Philippines	T52_15	Propiconazole	2.329	0.287	4.372	4	0.451	1.445	3.214	**5.025**
Philippines	T52_16	Difenoconazole	3.185	1.143	5.227	1	0.901	1.416	4.954	**9.095**
Philippines	T52_16	Epoxiconazole	2.786	0.744	4.828	1	0.901	1.017	4.555	**6.897**
Philippines	T52_16	Propiconazole	2.805	0.763	4.847	1	0.901	1.036	4.574	**6.989**
Philippines	T52_17	Difenoconazole				0				**>10.24**
Philippines	T52_17	Epoxiconazole				0				**>10.24**
Philippines	T52_17	Propiconazole				0				**>10.24**
Philippines	T52_18	Difenoconazole				0				**>10.24**
Philippines	T52_18	Epoxiconazole	3.095	1.053	5.137	1	0.901	1.326	4.864	**8.544**
Philippines	T52_18	Propiconazole	2.538	0.495	4.580	1	0.901	0.769	4.306	**5.807**
Philippines	T52_19	Difenoconazole	2.869	0.827	4.912	1	0.901	1.101	4.638	**7.307**
Philippines	T52_19	Epoxiconazole	3.016	0.974	5.058	1	0.901	1.247	4.785	**8.089**
Philippines	T52_19	Propiconazole	1.668	-0.374	3.710	1	0.901	-0.101	3.437	**3.178**
Philippines	T52_2	Difenoconazole	3.327	1.285	5.369	1	0.901	1.558	5.096	**10.035**
Philippines	T52_2	Epoxiconazole	1.285	-0.758	3.327	2	0.637	0.034	2.535	**2.436**
Philippines	T52_2	Propiconazole	3.136	1.094	5.178	2	0.637	1.885	4.387	**8.791**
Philippines	T52_20	Difenoconazole	2.609	0.567	4.652	1	0.901	0.840	4.378	**6.101**
Philippines	T52_20	Epoxiconazole	2.298	0.255	4.340	1	0.901	0.529	4.066	**4.917**
Philippines	T52_20	Propiconazole	2.566	0.524	4.608	1	0.901	0.797	4.335	**5.922**
Philippines	T52_21	Difenoconazole	2.904	0.862	4.946	1	0.901	1.135	4.673	**7.485**
Philippines	T52_21	Epoxiconazole				0				**>10.24**
Philippines	T52_21	Propiconazole	2.912	0.869	4.954	1	0.901	1.143	4.681	**7.525**
Philippines	T52_22	Difenoconazole	2.815	0.772	4.857	4	0.451	1.930	3.699	**7.035**
Philippines	T52_22	Epoxiconazole	1.735	-0.308	3.777	6	0.368	1.012	2.457	**3.328**
Philippines	T52_22	Propiconazole	3.067	1.024	5.109	5	0.403	2.276	3.858	**8.379**
Philippines	T52_23	Difenoconazole	-0.064	-2.107	1.978	3	0.520	-1.085	0.957	**0.956**
Philippines	T52_23	Epoxiconazole	0.115	-1.928	2.157	3	0.520	-0.907	1.136	**1.083**
Philippines	T52_23	Propiconazole	-0.558	-2.600	1.484	2	0.637	-1.809	0.693	**0.679**
Philippines	T52_3	Difenoconazole	3.255	1.212	5.297	1	0.901	1.486	5.023	**9.544**
Philippines	T52_3	Epoxiconazole	2.678	0.636	4.721	1	0.901	0.910	4.447	**6.402**
Philippines	T52_3	Propiconazole	0.693	-1.350	2.735	1	0.901	-1.076	2.462	**1.616**
Philippines	T52_36	Difenoconazole	1.438	-0.605	3.480	1	0.901	-0.331	3.207	**2.709**
Philippines	T52_36	Epoxiconazole	1.295	-0.748	3.337	1	0.901	-0.474	3.063	**2.453**
Philippines	T52_36	Propiconazole	0.218	-1.825	2.260	1	0.901	-1.551	1.987	**1.163**
Philippines	T52_4	Difenoconazole				0				**>10.24**
Philippines	T52_4	Epoxiconazole				0				**>10.24**

Country	Isolate	Fungicide	[2]Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Philippines	T52_4	Propiconazole				0				**>10.24**
Philippines	T52_5	Difenoconazole	3.353	1.311	5.396	1	0.901	1.585	5.122	**10.220**
Philippines	T52_5	Epoxiconazole	3.085	1.042	5.127	2	0.637	1.834	4.335	**8.484**
Philippines	T52_5	Propiconazole	2.694	0.651	4.736	3	0.520	1.673	3.715	**6.470**
Philippines	T52_6	Difenoconazole				0				**>10.24**
Philippines	T52_6	Epoxiconazole	2.874	0.832	4.916	1	0.901	1.105	4.643	**7.331**
Philippines	T52_6	Propiconazole				0				**>10.24**
Philippines	T52_7	Difenoconazole	2.539	0.496	4.581	1	0.901	0.770	4.307	**5.810**
Philippines	T52_7	Epoxiconazole	2.689	0.646	4.731	1	0.901	0.920	4.458	**6.448**
Philippines	T52_7	Propiconazole				0				**>10.24**
Philippines	T52_8	Difenoconazole	2.920	0.877	4.962	3	0.520	1.898	3.941	**7.567**
Philippines	T52_8	Epoxiconazole	1.343	-0.699	3.386	3	0.520	0.322	2.365	**2.538**
Philippines	T52_8	Propiconazole	1.974	-0.068	4.016	3	0.520	0.953	2.995	**3.929**
Philippines	T52_9	Difenoconazole				0				**>10.24**
Philippines	T52_9	Epoxiconazole				0				**>10.24**
Philippines	T52_9	Propiconazole				0				**>10.24**
Colombia	Tamaca_1	Difenoconazole	0.165	-1.877	2.208	1	0.901	-1.603	1.934	**1.121**
Colombia	Tamaca_1	Epoxiconazole	-1.102	-3.145	0.940	1	0.901	-2.871	0.666	**0.466**
Colombia	Tamaca_1	Propiconazole	0.387	-1.655	2.430	1	0.901	-1.381	2.156	**1.308**
Colombia	Teresa_1	Difenoconazole	2.790	0.748	4.832	1	0.901	1.021	4.559	**6.916**
Colombia	Teresa_1	Epoxiconazole	-3.510	-5.553	-1.468	1	0.901	-5.279	-1.742	**0.088**
Colombia	Teresa_1	Propiconazole	0.634	-1.408	2.676	1	0.901	-1.135	2.403	**1.552**
Colombia	Teresa_2	Difenoconazole	-0.313	-2.356	1.729	1	0.901	-2.082	1.455	**0.805**
Colombia	Teresa_2	Epoxiconazole	-0.914	-2.956	1.129	1	0.901	-2.683	0.855	**0.531**
Colombia	Teresa_2	Propiconazole	0.130	-1.913	2.172	1	0.901	-1.639	1.898	**1.094**
Colombia	Teresa_3	Difenoconazole	-0.534	-2.576	1.508	3	0.520	-1.555	0.487	**0.691**
Colombia	Teresa_3	Epoxiconazole	0.333	-1.710	2.375	3	0.520	-0.688	1.354	**1.259**
Colombia	Teresa_3	Propiconazole	1.954	-0.089	3.996	3	0.520	0.933	2.975	**3.874**
Colombia	Toscana_12	Difenoconazole	2.554	0.511	4.596	1	0.901	0.785	4.322	**5.871**
Colombia	Toscana_12	Epoxiconazole	0.910	-1.132	2.952	1	0.901	-0.859	2.679	**1.879**
Colombia	Toscana_12	Propiconazole	2.562	0.520	4.604	1	0.901	0.793	4.331	**5.906**
Colombia	Toscana_2	Difenoconazole	1.833	-0.209	3.876	1	0.901	0.065	3.602	**3.564**
Colombia	Toscana_2	Epoxiconazole	1.905	-0.138	3.947	1	0.901	0.136	3.673	**3.744**
Colombia	Toscana_2	Propiconazole	2.684	0.642	4.727	1	0.901	0.915	4.453	**6.427**
Colombia	Toscana_3	Difenoconazole	1.113	-0.929	3.156	1	0.901	-0.656	2.882	**2.163**
Colombia	Toscana_3	Epoxiconazole	-0.004	-2.047	2.038	1	0.901	-1.773	1.765	**0.997**
Colombia	Toscana_3	Propiconazole	0.938	-1.104	2.981	1	0.901	-0.830	2.707	**1.916**
Colombia	Toscana_4	Difenoconazole	-1.668	-3.710	0.375	1	0.901	-3.436	0.101	**0.315**

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Colombia	Toscana_4	Epoxiconazole	-3.667	-5.709	-1.625	1	0.901	-5.436	-1.898	**0.079**
Colombia	Toscana_4	Propiconazole	-0.678	-2.721	1.364	1	0.901	-2.447	1.090	**0.625**
Colombia	Toscana_5	Difenoconazole	1.264	-0.778	3.306	1	0.901	-0.505	3.033	**2.402**
Colombia	Toscana_5	Epoxiconazole	0.231	-1.811	2.273	1	0.901	-1.538	2.000	**1.174**
Colombia	Toscana_5	Propiconazole	2.777	0.735	4.820	1	0.901	1.009	4.546	**6.856**
Colombia	Toscana_6	Difenoconazole	2.207	0.165	4.249	1	0.901	0.438	3.976	**4.617**
Colombia	Toscana_6	Epoxiconazole	0.001	-2.041	2.044	1	0.901	-1.767	1.770	**1.001**
Colombia	Toscana_6	Propiconazole	0.911	-1.131	2.954	1	0.901	-0.858	2.680	**1.881**
Colombia	Toscana_7	Difenoconazole	1.125	-0.918	3.167	1	0.901	-0.644	2.893	**2.181**
Colombia	Toscana_7	Epoxiconazole	-0.654	-2.696	1.388	1	0.901	-2.423	1.115	**0.636**
Colombia	Toscana_7	Propiconazole	1.021	-1.022	3.063	1	0.901	-0.748	2.789	**2.029**
Colombia	Toscana_8	Difenoconazole	1.950	-0.092	3.993	4	0.451	1.066	2.835	**3.864**
Colombia	Toscana_8	Epoxiconazole	1.081	-0.961	3.124	4	0.451	0.197	1.966	**2.116**
Colombia	Toscana_8	Propiconazole	2.356	0.314	4.399	4	0.451	1.472	3.241	**5.120**
Philippines	U22_1	Difenoconazole	0.385	-1.657	2.428	2	0.637	-0.865	1.636	**1.306**
Philippines	U22_1	Epoxiconazole	0.449	-1.593	2.491	2	0.637	-0.802	1.700	**1.365**
Philippines	U22_1	Propiconazole	0.772	-1.270	2.814	2	0.637	-0.479	2.023	**1.708**
Philippines	U22_10	Difenoconazole	0.935	-1.107	2.978	1	0.901	-0.834	2.704	**1.912**
Philippines	U22_10	Epoxiconazole	0.603	-1.440	2.645	1	0.901	-1.166	2.371	**1.518**
Philippines	U22_10	Propiconazole	0.586	-1.457	2.628	1	0.901	-1.183	2.354	**1.501**
Philippines	U22_11	Difenoconazole	2.389	0.347	4.432	1	0.901	0.620	4.158	**5.239**
Philippines	U22_11	Epoxiconazole	0.350	-1.692	2.392	1	0.901	-1.419	2.119	**1.274**
Philippines	U22_11	Propiconazole	1.315	-0.727	3.357	1	0.901	-0.454	3.084	**2.488**
Philippines	U22_12	Difenoconazole	1.485	-0.558	3.527	1	0.901	-0.284	3.254	**2.799**
Philippines	U22_12	Epoxiconazole	1.371	-0.671	3.413	1	0.901	-0.398	3.140	**2.586**
Philippines	U22_12	Propiconazole	1.428	-0.614	3.470	1	0.901	-0.341	3.197	**2.691**
Philippines	U22_13	Difenoconazole	2.650	0.608	4.692	1	0.901	0.881	4.419	**6.277**
Philippines	U22_13	Epoxiconazole	2.443	0.401	4.486	1	0.901	0.675	4.212	**5.439**
Philippines	U22_13	Propiconazole	0.539	-1.503	2.582	1	0.901	-1.230	2.308	**1.453**
Philippines	U22_14	Difenoconazole	-1.134	-3.176	0.909	1	0.901	-2.902	0.635	**0.456**
Philippines	U22_14	Epoxiconazole	-1.709	-3.751	0.334	1	0.901	-3.477	0.060	**0.306**
Philippines	U22_14	Propiconazole	-0.812	-2.854	1.230	1	0.901	-2.581	0.957	**0.570**
Philippines	U22_15	Difenoconazole	0.370	-1.673	2.412	1	0.901	-1.399	2.138	**1.292**
Philippines	U22_15	Epoxiconazole	-0.493	-2.535	1.549	1	0.901	-2.262	1.276	**0.711**
Philippines	U22_15	Propiconazole	0.938	-1.104	2.980	1	0.901	-0.831	2.707	**1.916**
Philippines	U22_16	Difenoconazole	2.790	0.748	4.832	1	0.901	1.021	4.559	**6.916**
Philippines	U22_16	Epoxiconazole	2.168	0.125	4.210	1	0.901	0.399	3.936	**4.493**
Philippines	U22_16	Propiconazole	1.417	-0.625	3.460	1	0.901	-0.352	3.186	**2.671**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Philippines	U22_17	Difenoconazole	-0.501	-2.544	1.541	1	0.901	-2.270	1.267	**0.706**
Philippines	U22_17	Epoxiconazole	-0.875	-2.917	1.168	1	0.901	-2.644	0.894	**0.545**
Philippines	U22_17	Propiconazole	0.364	-1.678	2.407	1	0.901	-1.405	2.133	**1.287**
Philippines	U22_18	Difenoconazole	1.764	-0.278	3.806	1	0.901	-0.005	3.533	**3.397**
Philippines	U22_18	Epoxiconazole	1.250	-0.792	3.292	1	0.901	-0.519	3.019	**2.379**
Philippines	U22_18	Propiconazole	0.715	-1.328	2.757	1	0.901	-1.054	2.483	**1.641**
Philippines	U22_19	Difenoconazole	-1.072	-3.114	0.971	1	0.901	-2.840	0.697	**0.476**
Philippines	U22_19	Epoxiconazole	-1.731	-3.773	0.311	1	0.901	-3.500	0.038	**0.301**
Philippines	U22_19	Propiconazole	-1.186	-3.229	0.856	1	0.901	-2.955	0.582	**0.439**
Philippines	U22_2	Difenoconazole	1.067	-0.976	3.109	3	0.520	0.046	2.088	**2.095**
Philippines	U22_2	Epoxiconazole	0.663	-1.380	2.705	3	0.520	-0.359	1.684	**1.583**
Philippines	U22_2	Propiconazole	1.377	-0.665	3.420	3	0.520	0.356	2.399	**2.598**
Philippines	U22_20	Difenoconazole	2.201	0.158	4.243	1	0.901	0.432	3.970	**4.598**
Philippines	U22_20	Epoxiconazole	1.826	-0.216	3.869	1	0.901	0.058	3.595	**3.546**
Philippines	U22_20	Propiconazole	0.624	-1.419	2.666	1	0.901	-1.145	2.393	**1.541**
Philippines	U22_21	Difenoconazole	1.357	-0.685	3.400	1	0.901	-0.411	3.126	**2.562**
Philippines	U22_21	Epoxiconazole	1.426	-0.616	3.469	1	0.901	-0.343	3.195	**2.687**
Philippines	U22_21	Propiconazole	0.853	-1.189	2.895	1	0.901	-0.916	2.622	**1.806**
Philippines	U22_22	Difenoconazole	0.607	-1.435	2.649	1	0.901	-1.162	2.376	**1.523**
Philippines	U22_22	Epoxiconazole	0.633	-1.409	2.676	1	0.901	-1.136	2.402	**1.551**
Philippines	U22_22	Propiconazole	0.693	-1.350	2.735	1	0.901	-1.076	2.462	**1.616**
Philippines	U22_3	Difenoconazole	-0.356	-2.398	1.686	3	0.520	-1.377	0.665	**0.781**
Philippines	U22_3	Epoxiconazole	-1.241	-3.284	0.801	3	0.520	-2.262	-0.220	**0.423**
Philippines	U22_3	Propiconazole	-0.888	-2.930	1.154	3	0.520	-1.909	0.133	**0.540**
Philippines	U22_4	Difenoconazole	-1.549	-3.592	0.493	3	0.520	-2.570	-0.528	**0.342**
Philippines	U22_4	Epoxiconazole	-1.701	-3.743	0.341	3	0.520	-2.722	-0.680	**0.308**
Philippines	U22_4	Propiconazole	-0.418	-2.460	1.625	3	0.520	-1.439	0.604	**0.749**
Philippines	U22_5	Difenoconazole	-1.508	-3.550	0.534	3	0.520	-2.529	-0.487	**0.352**
Philippines	U22_5	Epoxiconazole	-1.690	-3.732	0.353	3	0.520	-2.711	-0.668	**0.310**
Philippines	U22_5	Propiconazole	-0.849	-2.892	1.193	3	0.520	-1.871	0.172	**0.555**
Philippines	U22_6	Difenoconazole	-1.012	-3.054	1.031	3	0.520	-2.033	0.009	**0.496**
Philippines	U22_6	Epoxiconazole	-1.462	-3.504	0.581	3	0.520	-2.483	-0.441	**0.363**
Philippines	U22_6	Propiconazole	-0.257	-2.299	1.786	3	0.520	-1.278	0.764	**0.837**
Philippines	U22_7	Difenoconazole	0.199	-1.843	2.242	1	0.901	-1.570	1.968	**1.148**
Philippines	U22_7	Epoxiconazole	-0.334	-2.376	1.708	1	0.901	-2.103	1.435	**0.793**
Philippines	U22_7	Propiconazole	0.454	-1.589	2.496	1	0.901	-1.315	2.222	**1.369**
Philippines	U22_8	Difenoconazole	2.816	0.773	4.858	1	0.901	1.047	4.584	**7.040**
Philippines	U22_8	Epoxiconazole	2.738	0.696	4.781	1	0.901	0.970	4.507	**6.674**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Philippines	U22_8	Propiconazole	2.517	0.474	4.559	1	0.901	0.748	4.285	**5.722**
Philippines	U22_9	Difenoconazole	-0.638	-2.681	1.404	1	0.901	-2.407	1.131	**0.643**
Philippines	U22_9	Epoxiconazole	-1.394	-3.437	0.648	1	0.901	-3.163	0.374	**0.380**
Philippines	U22_9	Propiconazole	0.233	-1.810	2.275	1	0.901	-1.536	2.001	**1.175**
Colombia	Universalia_1	Difenoconazole	-3.646	-5.688	-1.603	1	0.901	-5.414	-1.877	**0.080**
Colombia	Universalia_1	Epoxiconazole	-5.279	-7.321	-3.236	1	0.901	-7.047	-3.510	**0.026**
Colombia	Universalia_1	Propiconazole	-2.090	-4.133	-0.048	1	0.901	-3.859	-0.321	**0.235**
Colombia	Universalia_2	Difenoconazole	-5.315	-7.357	-3.273	1	0.901	-7.084	-3.546	**0.025**
Colombia	Universalia_2	Epoxiconazole	-1.665	-3.708	0.377	1	0.901	-3.434	0.104	**0.315**
Colombia	Universalia_2	Propiconazole	1.311	-0.731	3.353	1	0.901	-0.458	3.080	**2.481**
Colombia	Universalia_3	Difenoconazole	2.804	0.762	4.847	2	0.637	1.554	4.055	**6.985**
Colombia	Universalia_3	Epoxiconazole	1.479	-0.564	3.521	3	0.520	0.458	2.500	**2.787**
Colombia	Universalia_3	Propiconazole	0.995	-1.047	3.037	2	0.637	-0.256	2.246	**1.993**
Colombia	Vega_1	Difenoconazole	1.015	-1.027	3.058	1	0.901	-0.754	2.784	**2.021**
Colombia	Vega_1	Epoxiconazole	0.716	-1.326	2.759	1	0.901	-1.052	2.485	**1.643**
Colombia	Vega_1	Propiconazole	2.497	0.454	4.539	1	0.901	0.728	4.266	**5.644**
Colombia	Victoria	Difenoconazole	2.199	0.157	4.242	1	0.901	0.431	3.968	**4.593**
Colombia	Victoria	Epoxiconazole	1.846	-0.197	3.888	1	0.901	0.077	3.614	**3.594**
Colombia	Victoria	Propiconazole	-0.437	-2.480	1.605	1	0.901	-2.206	1.332	**0.739**
Cameroon	X02_4	Difenoconazole	-7.663	-9.706	-5.621	3	0.520	-8.685	-6.642	**0.005**
Cameroon	X02_4	Epoxiconazole	-6.968	-9.010	-4.925	3	0.520	-7.989	-5.946	**0.008**
Cameroon	X02_4	Propiconazole	-6.547	-8.589	-4.504	3	0.520	-7.568	-5.525	**0.011**
Cameroon	X03_2	Difenoconazole	-7.644	-9.686	-5.601	2	0.637	-8.895	-6.393	**0.005**
Cameroon	X03_2	Epoxiconazole	-7.437	-9.480	-5.395	3	0.520	-8.459	-6.416	**0.006**
Cameroon	X03_2	Propiconazole	-6.835	-8.877	-4.793	3	0.520	-7.856	-5.814	**0.009**
Cameroon	X04_2	Difenoconazole	-7.966	-10.008	-5.923	3	0.520	-8.987	-6.945	**0.004**
Cameroon	X04_2	Epoxiconazole	-7.199	-9.242	-5.157	3	0.520	-8.221	-6.178	**0.007**
Cameroon	X04_2	Propiconazole	-6.418	-8.460	-4.375	3	0.520	-7.439	-5.397	**0.012**
Cameroon	X04_5	Difenoconazole	-7.562	-9.604	-5.520	2	0.637	-8.813	-6.311	**0.005**
Cameroon	X04_5	Epoxiconazole	-6.609	-8.651	-4.566	2	0.637	-7.859	-5.358	**0.010**
Cameroon	X04_5	Propiconazole	-6.119	-8.161	-4.077	2	0.637	-7.370	-4.868	**0.014**
Cameroon	X05_3	Difenoconazole	-7.163	-9.205	-5.120	3	0.520	-8.184	-6.141	**0.007**
Cameroon	X05_3	Epoxiconazole	-6.844	-8.886	-4.801	3	0.520	-7.865	-5.822	**0.009**
Cameroon	X05_3	Propiconazole	-6.264	-8.306	-4.221	3	0.520	-7.285	-5.242	**0.013**
Cameroon	X05_5	Difenoconazole	-7.506	-9.548	-5.463	4	0.451	-8.390	-6.621	**0.006**
Cameroon	X05_5	Epoxiconazole	-7.065	-9.107	-5.022	4	0.451	-7.949	-6.180	**0.007**
Cameroon	X05_5	Propiconazole	-6.011	-8.053	-3.968	4	0.451	-6.895	-5.126	**0.016**
Cameroon	X07_2	Difenoconazole	-7.270	-9.312	-5.227	2	0.637	-8.520	-6.019	**0.006**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Cameroon	X07_2	Epoxiconazole	-6.550	-8.592	-4.507	3	0.520	-7.571	-5.529	**0.011**
Cameroon	X07_2	Propiconazole	-6.004	-8.047	-3.962	3	0.520	-7.026	-4.983	**0.016**
Cameroon	X08_1	Difenoconazole	-7.644	-9.686	-5.601	2	0.637	-8.895	-6.393	**0.005**
Cameroon	X08_1	Epoxiconazole	-6.060	-8.103	-4.018	3	0.520	-7.081	-5.039	**0.015**
Cameroon	X08_1	Propiconazole	-6.015	-8.058	-3.973	3	0.520	-7.036	-4.994	**0.015**
Cameroon	X08_2	Difenoconazole	-7.644	-9.686	-5.601	2	0.637	-8.895	-6.393	**0.005**
Cameroon	X08_2	Epoxiconazole	-6.835	-8.877	-4.793	3	0.520	-7.856	-5.814	**0.009**
Cameroon	X08_2	Propiconazole	-6.117	-8.159	-4.074	3	0.520	-7.138	-5.096	**0.014**
Cameroon	X13_3	Difenoconazole	-7.576	-9.618	-5.533	3	0.520	-8.597	-6.555	**0.005**
Cameroon	X13_3	Epoxiconazole	-6.676	-8.719	-4.634	3	0.520	-7.697	-5.655	**0.010**
Cameroon	X13_3	Propiconazole	-6.428	-8.470	-4.385	3	0.520	-7.449	-5.406	**0.012**
Cameroon	X14_3	Difenoconazole	-7.512	-9.555	-5.470	2	0.637	-8.763	-6.262	**0.005**
Cameroon	X14_3	Epoxiconazole	-6.815	-8.858	-4.773	3	0.520	-7.837	-5.794	**0.009**
Cameroon	X14_3	Propiconazole	-5.968	-8.010	-3.925	3	0.520	-6.989	-4.946	**0.016**
Cameroon	X14_4	Difenoconazole	-7.663	-9.706	-5.621	3	0.520	-8.685	-6.642	**0.005**
Cameroon	X14_4	Epoxiconazole	-6.745	-8.788	-4.703	3	0.520	-7.766	-5.724	**0.009**
Cameroon	X14_4	Propiconazole	-6.366	-8.408	-4.324	3	0.520	-7.387	-5.345	**0.012**
Cameroon	X14_5	Difenoconazole	-7.104	-9.146	-5.062	3	0.520	-8.125	-6.083	**0.007**
Cameroon	X14_5	Epoxiconazole	-5.340	-7.382	-3.297	3	0.520	-6.361	-4.319	**0.025**
Cameroon	X14_5	Propiconazole	-5.152	-7.194	-3.109	3	0.520	-6.173	-4.131	**0.028**
Cameroon	X16_1	Difenoconazole	-7.413	-9.455	-5.371	3	0.520	-8.434	-6.392	**0.006**
Cameroon	X16_1	Epoxiconazole	-6.260	-8.302	-4.218	3	0.520	-7.281	-5.239	**0.013**
Cameroon	X16_1	Propiconazole	-5.858	-7.901	-3.816	3	0.520	-6.880	-4.837	**0.017**
Cameroon	X16_3	Difenoconazole	-6.629	-8.671	-4.586	3	0.520	-7.650	-5.607	**0.010**
Cameroon	X16_3	Epoxiconazole	-4.915	-6.958	-2.873	3	0.520	-5.936	-3.894	**0.033**
Cameroon	X16_3	Propiconazole	-4.269	-6.311	-2.227	3	0.520	-5.290	-3.248	**0.052**
Cameroon	X18_10	Difenoconazole	-7.792	-9.834	-5.749	2	0.637	-9.042	-6.541	**0.005**
Cameroon	X18_10	Epoxiconazole	-6.945	-8.988	-4.903	2	0.637	-8.196	-5.694	**0.008**
Cameroon	X18_10	Propiconazole	-6.592	-8.634	-4.549	3	0.520	-7.613	-5.571	**0.010**
Cameroon	X18_5	Difenoconazole	-7.170	-9.212	-5.127	3	0.520	-8.191	-6.149	**0.007**
Cameroon	X18_5	Epoxiconazole	-5.869	-7.912	-3.827	3	0.520	-6.890	-4.848	**0.017**
Cameroon	X18_5	Propiconazole	-5.652	-7.694	-3.609	3	0.520	-6.673	-4.630	**0.020**
Cameroon	X18_7	Difenoconazole	-7.134	-9.176	-5.091	2	0.637	-8.385	-5.883	**0.007**
Cameroon	X18_7	Epoxiconazole	-6.606	-8.649	-4.564	3	0.520	-7.627	-5.585	**0.010**
Cameroon	X18_7	Propiconazole	-5.607	-7.650	-3.565	3	0.520	-6.629	-4.586	**0.021**
Cameroon	X18_8	Difenoconazole	-7.341	-9.383	-5.299	3	0.520	-8.362	-6.320	**0.006**
Cameroon	X18_8	Epoxiconazole	-6.613	-8.655	-4.571	3	0.520	-7.634	-5.592	**0.010**
Cameroon	X18_8	Propiconazole	-5.849	-7.892	-3.807	3	0.520	-6.870	-4.828	**0.017**

Country	Isolate	Fungicide	^{2}Log(m)	Lsed	Used	(n)	Sem	LMCI	UMCI	EC_{50}
Cameroon	X19_1	Difenoconazole	-7.291	-9.333	-5.248	3	0.520	-8.312	-6.270	**0.006**
Cameroon	X19_1	Epoxiconazole	-6.446	-8.488	-4.403	3	0.520	-7.467	-5.425	**0.011**
Cameroon	X19_1	Propiconazole	-5.961	-8.003	-3.918	3	0.520	-6.982	-4.940	**0.016**
Cameroon	X19_3	Difenoconazole	-7.644	-9.686	-5.601	1	0.901	-9.413	-5.875	**0.005**
Cameroon	X19_3	Epoxiconazole	-7.663	-9.706	-5.621	3	0.520	-8.685	-6.642	**0.005**
Cameroon	X19_3	Propiconazole	-7.556	-9.599	-5.514	3	0.520	-8.577	-6.535	**0.005**
Cameroon	X23_2	Difenoconazole	-7.519	-9.561	-5.476	2	0.637	-8.769	-6.268	**0.005**
Cameroon	X23_2	Epoxiconazole	-7.242	-9.284	-5.199	3	0.520	-8.263	-6.221	**0.007**
Cameroon	X23_2	Propiconazole	-6.237	-8.279	-4.195	3	0.520	-7.258	-5.216	**0.013**
Cameroon	X23_3	Difenoconazole	-7.151	-9.193	-5.109	3	0.520	-8.172	-6.130	**0.007**
Cameroon	X23_3	Epoxiconazole	-5.791	-7.833	-3.749	3	0.520	-6.812	-4.770	**0.018**
Cameroon	X23_3	Propiconazole	-5.415	-7.458	-3.373	3	0.520	-6.436	-4.394	**0.023**
Cameroon	X24_2	Difenoconazole	-7.412	-9.454	-5.369	3	0.520	-8.433	-6.391	**0.006**
Cameroon	X24_2	Epoxiconazole	-6.628	-8.671	-4.586	3	0.520	-7.649	-5.607	**0.010**
Cameroon	X24_2	Propiconazole	-6.120	-8.162	-4.077	3	0.520	-7.141	-5.099	**0.014**
Cameroon	X26_7	Difenoconazole	-7.184	-9.227	-5.142	3	0.520	-8.206	-6.163	**0.007**
Cameroon	X26_7	Epoxiconazole	-6.487	-8.529	-4.444	3	0.520	-7.508	-5.466	**0.011**
Cameroon	X26_7	Propiconazole	-5.858	-7.900	-3.815	3	0.520	-6.879	-4.836	**0.017**
Costa Rica	ZentM1_1	Difenoconazole	0.767	-1.275	2.809	3	0.520	-0.254	1.788	**1.702**
Costa Rica	ZentM1_1	Epoxiconazole	0.278	-1.764	2.321	3	0.520	-0.743	1.299	**1.213**
Costa Rica	ZentM1_1	Propiconazole	1.575	-0.468	3.617	3	0.520	0.554	2.596	**2.979**
Costa Rica	ZentM1_2	Difenoconazole	-0.848	-2.891	1.194	3	0.520	-1.870	0.173	**0.555**
Costa Rica	ZentM1_2	Epoxiconazole	-1.206	-3.249	0.836	3	0.520	-2.227	-0.185	**0.433**
Costa Rica	ZentM1_2	Propiconazole	-1.561	-3.603	0.482	3	0.520	-2.582	-0.540	**0.339**
Costa Rica	ZentM2_2	Difenoconazole	0.577	-1.466	2.619	3	0.520	-0.445	1.598	**1.491**
Costa Rica	ZentM2_2	Epoxiconazole	0.078	-1.964	2.120	3	0.520	-0.943	1.099	**1.055**
Costa Rica	ZentM2_2	Propiconazole	0.321	-1.721	2.364	3	0.520	-0.700	1.343	**1.250**
Colombia	Zurrambay_1	Difenoconazole	-4.212	-6.254	-2.169	1	0.901	-5.981	-2.443	**0.054**
Colombia	Zurrambay_1	Epoxiconazole	-4.818	-6.860	-2.775	1	0.901	-6.586	-3.049	**0.035**
Colombia	Zurrambay_1	Propiconazole	-3.603	-5.645	-1.561	1	0.901	-5.372	-1.834	**0.082**
Colombia	Zurrambay_2	Difenoconazole	-5.990	-8.033	-3.948	1	0.901	-7.759	-4.222	**0.016**
Colombia	Zurrambay_2	Epoxiconazole	-4.760	-6.803	-2.718	1	0.901	-6.529	-2.991	**0.037**
Colombia	Zurrambay_2	Propiconazole	-4.597	-6.640	-2.555	1	0.901	-6.366	-2.829	**0.041**
Colombia	Zurrambay_3	Difenoconazole	-5.651	-7.694	-3.609	1	0.901	-7.420	-3.883	**0.020**
Colombia	Zurrambay_3	Epoxiconazole	-4.652	-6.694	-2.609	1	0.901	-6.421	-2.883	**0.040**
Colombia	Zurrambay_3	Propiconazole	-4.010	-6.052	-1.968	1	0.901	-5.779	-2.241	**0.062**

Table S2. Description of the *Pseudocercospora fijiensis* population EC_{50} values. Minimum, maximum, average and standard deviation per country and the percentage of the sensitivity trait are indicated. The sensitivity trait was characterized by arbitrary thresholds. Average EC_{50} values higher than 1 $mg.L^{-1}$were labelled "resistant", 0.1 to 0.99 $mg.L^{-1}$ "tolerant" and lower than 0.1 $mg.L^{-1}$ as "sensitive".

Fungicide		Difenoconazole				Epoxiconazole				Propiconazole			
Country (Cycles DMI/total)	Resistance Categories*	Mini. Value	Max. Value	Average (SD)	Percentage	Mini. Value	Max. Value	Average (SD)	Percentage	Mini. Value	Max. Value	Average (SD)	Percentage
Costa Rica 7/56	S	0	0	0	0%	0	0	0	0%	0	0	0	0%
	T	0.526	0.894	0.725 (0.170)	1.87%	0.403	0.993	0.675 (0.235)	2.80%	0.339	0.339	0.339 (0)	0.94%
	R	1.491	20.925	6.172 (3.214)	98.13%	1.016	10.054	4.129 (2.186)	97.20%	1.172	18.414	5.814 (3.462)	99.07%
Colombia 7/32	S	0.005	0.080	0.02 (0.002)	15.31%	0.014	0.088	0.03 (0.022)	16.33%	0.016	0.082	0.038 (0.021)	13.27%
	T	0.123	0.940	0.538 (0.276)	13.27%	0.118	0.997	0.555 (0.240)	34.69%	0.235	0.983	0.670 (0.246)	17.35%
	R	1.121	10.418	4.571 (2.415)	71.43%	1.001	8.411	2.913 (1.926)	48.98%	1.079	13.653	3.806 (2.209)	69.39%
Cameroon 7/45	S	0.004	0.096	0.011 (0.018)	36.96%	0.004	0.097	0.018 (0.02)	38.04%	0.005	0.052	0.017 (0.009)	33.70%
	T	0.107	0.981	0.512 (0.196)	44.57%	0.107	0.975	0.460 (0.21)	50.00%	0.188	0.986	0.622 (0.222)	27.17%
	R	1.011	6.663	1.748 (1.272)	18.48%	1.026	3.904	1.452 (0.792)	11.96%	1.039	7.816	1.658 (1.094)	39.13%
Dominican Republic ?	S	0.007	0.087	0.056 (0.03)	16%	0.012	0.09	0.045 (0.028)	20%	0.016	0.016	0.016 (0)	4%
	T	0.16	0.541	0.452 (0.259)	40%	0.155	0.59	0.598 (0.295)	52%	0.179	0.994	0.507 (0.297)	44%
	R	1.048	2.863	1.691 (0.510)	44%	1.165	1.819	1.402 (0.218)	28%	1.037	3.321	1.720 (0.704)	52%
Ecuador 13/30	S	0.004	0.084	0.026 (0.025)	29.7%	0.003	0.095	0.039 (0.029)	38.61%	0.004	0.086	0.027 (0.019)	24.75%
	T	0.105	0.945	0.337 (0.196)	53.47%	0.106	0.964	0.367 (0.232)	52.48%	0.103	0.328	0.433 (241)	53.47%
	R	1.079	5.152	1.848 (0.917)	16.83%	1.132	5.63	2.455 (1.484)	8.91%	1.006	5.351	1.928 (1.088)	21.78%
Guadalupe 6/10 (Low time exposure)	S	0.004	0.061	0.017 (0.013)	100%	0.005	0.083	0.020 (0.016)	100%	0.004	0.058	0.022 (0.015)	100%
	T	0	0	0	0%	0	0	0	0%	0	0	0	0%
	R	0	0	0	0%	0	0	0	0%	0	0	0	0%
Martinique 9/11 (Low time exposure)	S	0.004	0.086	0.022 (0.02)	100%	0.004	0.099	0.03 (0.022)	100%	0.005	0.086	0.022 (0.016)	100%
	T	0	0	0	0%	0	0	0	0%	0	0	0	0%
	R	0	0	0	0%	0	0	0	0%	0	0	0	0%
Philippines 12/54	S	0.006	0.099	0.066 (0.043)	3.06%	0.011	0.011	0.011 (0)	1.02%	0.017	0.099	0.058 (0.041)	2.04%
	T	0.168	0.956	0.510 (0.214)	38.78%	0.11	0.986	0.435 (0.238)	44.90%	0.166	0.954	0.683 (0.178)	25.51%
	R	1.029	10.22	4.351 (2.702)	58.16%	1.083	8.544	3.823 (2.295)	54.08%	1.029	12.518	3.079 (2.114)	72.45%

*Resistance categories: S=Sensitive, T=Tolerant, R=Resistant

Table S3. Different genotypes based on the CYP51 protein sequences of *Pseudocercospora fijiensis* isolates. The genotypes numbers are ordered based on the number of mutations. Average DMI EC50 values higher than 1 mg.L-1 are indicated in red, from 0.1 to 0.99 mg.L-1 in yellow and lower than 0.1 mg.L-1 in green. Predicted resistant mutations and the different promoter insertion are mark with different colours.

Genotype	Promoter element TCGTACGA	Substitutions found in the PfCYP51							Average EC_{50} value in mg.L^{-1}			(n)
		M1	M2	M3	M4	M5	M6	M7	Difenoconazole	Epoxiconazole	Propiconazole	
G1^ (model C86)	1								0.004	0.007	0.010	1
G2	1	V106D							0.022	0.024	0.048	32
G3	1	T18I	V106D						0.014	0.016	0.045	19
G4	1	T18I	A19E	V106D					0.007	0.009	0.011	2
G5	1	T18I	Y58F	V106D					*	*	0.079	1
G6	1	T18I	V106D	V116L					*	*	0.055	1
G7	1	T18I	V106D	I264T					0.072	0.035	0.027	1
G8	1	T18I	V106D	A381G					0.087	0.053	0.179	1
G9	1	T18I	V106D	R418G					0.007	0.012	0.016	1
G10 (model Bo_1)	1	T18I	V106D	A446S					0.006	0.011	0.017	1
G11	1	V106D	K171R	A446S					*	*	0.034	1
G12	3	V106D	A313G	D460V					0.798	0.62	1.188	45
G13	4	V106D	A313G	D460V					0.637	0.666	1.277	4
G14	1	V106D	A313G	G462D					0.055	0.058	0.188	1
G15	2	V106D	A313G	G462D					0.107	0.107	0.338	2
G16	1	V106D	A313G	Y463D					0.391	0.114	0.563	2
G17	3	V106D	A313G	Y463D					0.547	0.318	0.704	3
G18	4	V106D	A313G	Y463D					0.576	0.536	1.132	1
G19	3	V106D	A313G	Y463N					1.12	0.599	1.653	1

Genotype	Promoter element TCGTACGA	Substitutions found in the PfCYP51							Average EC_{50} value in $mg.L^{-1}$			(n)
		M1	M2	M3	M4	M5	M6	M7	Difenoconazole	Epoxiconazole	Propiconazole	
G20	4	V106D	A313G	Y463N					0.983	0.459	1.553	3
G21 (model Z4_16)	1	T18I	V106D	A381G	G462A				*	*	0.138	1
G22	3	T18I	V106D	Y136F	Y463D				*	*	0.192	2
G23	4	T18I	V106D	Y136F	Y463D				3.466	1.337	2.529	19
G24	6	T18I	V106D	Y136F	Y463D				5.744	2.32	3.777	4
G25	4	V106D	Y136F	A313G	G462D				6.663	3.904	7.816	1
G26	1	T18I	V106D	A313G	Y461N				0.304	0.172	0.611	2
G27	1	T18I	V106D	A313G	Y461D				*	*	0.147	1
G28	3	T18I	V106D	A313G	Y461D				1.376	1.132	2.823	1
G29	1	T18I	V106D	A313G	Y463D				*	*	1.049	2
G30	4	T18I	V106D	A313G	Y463D				1.306	1.365	1.708	1
G31	5	T18I	V106D	A313G	Y463D				2.371	1.401	1.96	4
G32	6	T18I	V106D	A313G	Y463D				2.02	1.423	1.949	16
G33	1	T18I	V106D	A313G	Y463H				0.289	0.19	0.452	12
G34	3	T18I	V106D	A313G	Y463H				0.94	0.589	0.983	2
G35	1	T18I	V106D	A313G	Y463N				0.252	0.24	0.624	19
G36	3	T18I	V106D	A313G	Y463N				1.478	0.759	2.236	3
G37	1	T18I	V106D	A313G	Y463S				*	*	0.207	3
G38	5	T18I	V106D	A313G	Y463S				1.411	0.786	1.344	1
G39	3	V106D	A313G	D460V	Y463N				1.405	1.1	1.764	1
G40	4	T18I	V106D	Y136F	A313G	Y461D			6.053	3.695	8.201	2

Genotype	Promoter element TCGTACGA	Substitutions found in the PfCYP51							Average EC_{50} value in $mg.L^{-1}$			(n)
		M1	M2	M3	M4	M5	M6	M7	Difenoconazole	Epoxiconazole	Propiconazole	
G41	6	T18I	V106D	Y136F	A381G	Y461D			*	0.27	3.16	1
G42	3	T18I	V106D	Y136F	A313G	Y463D			4.187	1.609	5.156	1
G43	4	T18I	V106D	Y136F	A313G	Y463D			8.535	2.882	8.585	4
G44 (model CaM10_6)	6	T18I	V106D	Y136F	A313G	Y463D			13.181	4.859	8.734	5
G45	5	T18I	V106D	Y136F	A313G	Y463S			7.91	4.448	5.588	1
G46	3	T18I	V106D	Y136F	A381G	Y463N			*	*	*	2
G47	5	T18I	V106D	Y136F	A381G	Y463N			*	*	*	1
G48	6	T18I	V106D	Y136F	A381G	Y463D			3.202	1.016	2.783	1
G49	1	T18I	V106D	A313G	A381G	Y463H			0.691	1.259	3.874	1
G50 (model CaM10_21)	6	T18I	V106D	H380N	A381G	Y463D			7.787	10.054	6.299	1
G51	6	T18I	V106D	H380N	A381G	Y463N			3.129	3.883	4.644	2
G52	1	T18I	V106D	A313G	A446S	Y461S			0.51	0.439	2.804	1
G53 (model M52_10)	1	V106D	A313G	A446S	D460E	Y461N			0.74	0.572	1.35	12
G54	6	T18I	V106D	Y136F	A313G	A381G	Y461D		6.042	4.41	5.069	1
G55	6	T18I	V106D	Y136F	V260L	A313G	Y463D		9.219	4.449	4.828	2
G56	1	T18I	V106D	K171R	A313G	A446S	Y461D		0.652	0.38	0.605	2
G57	1	T18I	V106D	K171R	A313G	A446S	Y461S		0.51	0.439	2.804	1
G58	1	V106D	K171R	A313G	A446S	D460E	Y461N		0.22	0.127	1.228	1
G59	4	T18I	V106D	A313G	A446S	D460E	Y461N		5.508	3.197	3.309	2
G60 (model M52_10)	1	T18I	I70M	D71E	V106D	A313G	A446S	Δ(Y461)	0.645	0.339	1.714	2

*Unknown data
^Reference strain CIRAD86

Table S4. Substitutions in the *Pseudocercospora fijiensis* CYP51 protein. The position of the reference codon, the reference sequence and the mutations in the *Pfcyp51* gene are indicated.

Substitution	Reference sequence	Position from start codon	Alternative mutations found	
T18I	ACA	53 bp	ATA	non
A19E	GCG	56 bp	GAG	non
Y58F	TAC	174 bp	TTC	non
I70M	ATC	210 bp	ATG	non
D71E	GAC	213 bp	GAA	non
V106D	GTC	318 bp	GAC	non
V116L	GTC	348 bp	CTC	non
Y136F	TAC	408 bp	TTC	TTT
K171R	AAA	513 bp	AGA	non
V260L	GTC	780 bp	CTC	non
I264T	ATC	792 bp	ACC	non
A313G	GCT	939 bp	GGT	non
H380N	CAT	1140 bp	AAT	non
A381G	GCT	1143 bp	GGT	non
R418G	CGA	1254 bp	GGA	non
A446S	GCA	1338 bp	TCA	non
D460E	GAT	1380 bp	GAA	non
D460V	GAT	1380 bp	GTT	non
Y461D	TAC	1383 bp	GAC	non
Y461N	TAC	1383 bp	AAC	non
Y461S	TAC	1383 bp	TCC	non
Δ(Y461)	TAC	1383 bp	---	non
G462A	GGC	1386 bp	GCC	non
G462D	GGC	1386 bp	GAC	non
Y463D	TAT	1389 bp	GAT	non
Y463H	TAT	1389 bp	CAT	non
Y463N	TAT	1389 bp	AAT	non
Y463S	TAT	1389 bp	TCT	non

Table S5. Characteristics of the amino acid changes in the enzyme 14α demethylase sequences of *Pseudocercospora fijiensis* isolates.

Position	Variant	Amino acid	Group	Characteristic		Hydrophobic Index*	
				pH2	pH7	pH2	pH7
T18	Wild type	Threonine	Neutral – polar side chain	Neutral	Neutral	13	13
I18	Mutant	Isoleucine	Hydrophobic aliphatic	Very Hydrophobic	Very Hydrophobic	100	99
A19	Wild type	Alanine	Hydrophobic aliphatic	Hydrophobic	Hydrophobic	47	41
E19	Mutant	Glutamic acid	Acidic	Neutral	Hydrophilic	8	-31
Y58	Wild type	Tyrosine	Hydrophobic aromatic	Hydrophobic	Hydrophobic	49	63
F58	Mutant	Phenylalanine	Hydrophobic aromatic	Very Hydrophobic	Very Hydrophobic	92	100
I70	Wild type	Isoleucine	Hydrophobic aliphatic	Very Hydrophobic	Very Hydrophobic	100	99
M70	Mutant	Methionine	Neutral – polar side chain	Very Hydrophobic	Very Hydrophobic	74	74
D71	Wild type	Aspartic Acid	Acidic	Neutral	Hydrophilic	-18	-55
E71	Mutant	Glutamic acid	Acidic	Neutral	Hydrophilic	8	-31
V106	Wild type	Valine	Hydrophobic aliphatic	Very Hydrophobic	Very Hydrophobic	79	76
D106	Mutant	Aspartic Acid	Acidic	Neutral	Hydrophilic	-18	-55
V116	Wild type	Valine	Hydrophobic aliphatic	Very Hydrophobic	Very Hydrophobic	79	76
L116	Mutant	Leucine	Hydrophobic aliphatic	Very Hydrophobic	Very Hydrophobic	100	97
Y136	Wild type	Tyrosine	Hydrophobic aromatic	Hydrophobic	Hydrophobic	49	63
F136	Mutant	Phenylalanine	Hydrophobic aromatic	Very Hydrophobic	Very Hydrophobic	92	100
K171	Wild type	Lysine	Basic	Hydrophilic	Hydrophilic	-37	-23
R171	Mutant	Arginine	Basic	Hydrophilic	Hydrophilic	-26	-14
V260	Wild type	Valine	Hydrophobic aliphatic	Very Hydrophobic	Very Hydrophobic	79	76
L260	Mutant	Leucine	Hydrophobic aliphatic	Very Hydrophobic	Very Hydrophobic	100	97
I264	Wild type	Isoleucine	Hydrophobic aliphatic	Very Hydrophobic	Very Hydrophobic	100	99
T264	Mutant	Threonine	Neutral – polar side chain	Neutral	Neutral	13	13
A313	Wild type	Alanine	Hydrophobic aliphatic	Hydrophobic	Hydrophobic	47	41
G313	Mutant	Glycine	Unique	Neutral	Neutral	0	0
H380	Wild type	Histidine	Basic	Hydrophobic	Neutral	-42	8
N380	Mutant	Asparagine	Neutral – polar side chain	Hydrophilic	Hydrophilic	-41	-28
A381	Wild type	Alanine	Hydrophobic aliphatic	Hydrophobic	Hydrophobic	47	41
G381	Mutant	Glycine	Unique	Neutral	Neutral	0	0
R418	Wild type	Arginine	Basic	Hydrophilic	Hydrophilic	-26	-14
G418	Mutant	Glycine	Unique	Neutral	Neutral	0	0
A446	Wild type	Alanine	Hydrophobic aliphatic	Hydrophobic	Hydrophobic	47	41
S446	Mutant	Serine	Neutral – polar side chain	Neutral	Neutral	-7	-5
D460	Wild type	Aspartic Acid	Acidic	Neutral	Hydrophilic	-18	-55
E460	Mutant	Glutamic acid	Acidic	Neutral	Hydrophilic	8	-31
V460	Mutant	Valine	Hydrophobic aliphatic	Very Hydrophobic	Very Hydrophobic	79	76
Y461	Wild type	Tyrosine	Hydrophobic aromatic	Hydrophobic	Hydrophobic	49	63
D461	Mutant	Aspartic Acid	Acidic	Neutral	Hydrophilic	-18	-55
N461	Mutant	Asparagine	Neutral – polar side chain	Hydrophilic	Hydrophilic	-41	-28
S461	Mutant	Serine	Neutral – polar side chain	Neutral	Neutral	-7	-5
Δ461	Mutant	non	non	non	non	non	Non
Y463	Wild type	Tyrosine	Hydrophobic aromatic	Hydrophobic	Hydrophobic	49	63
D463	Mutant	Aspartic Acid	Acidic	Neutral	Hydrophilic	-18	-55
N463	Mutant	Asparagine	Neutral – polar side chain	Hydrophilic	Hydrophilic	-41	-28
S463	Mutant	Serine	Neutral – polar side chain	Neutral	Neutral	-7	-5

*pH2 values normalized from Sereda et al 1994 (Sereda *et al.* 1994), pH7 values from Monera et al 1995 (Monera *et al.* 1995).

Table S6. YASARA CYP51 hybrid models quality Z-scores. The score includes floppy terminal tails. Values close to +1 are consider optimal (in green), negative values close to 0 are consider good (in Blue) and values close to -1 are consider satisfactory (in yellow).

	Hybrid models						
	C86	Bo_1	CaM10_21	Z14_16	M52_10	M52_22	CaM10_6
Check type	Quality Z-score						
Dihedrals	0.427	0.305	0.429	0.129	0.156	0.268	0.416
Packing 1D	-0.286	0.116	-0.279	0.163	0.04	0.117	-0.412
Packing 3D	-1.087	-1.083	-1.268	-1.132	-1.21	-1.057	-1.287
Overall	-0.555	-0.414	-0.636	-0.444	-0.524	-0.407	-0.699

Table S7. Comparison of distances of propiconazole docking simulated experiments in different *Pseudocercospora fijiensis* CYP51 models. Distance in angstrom (Å) of the amino acid positions that are present closer than 7Å to the nearest propiconazole atom. Amino acid substitutions in this area are mark in orange. All resistant models have substitutions around positions 461 to 463 (outside the substrate binding site). The specific substitutions are indicated below the model name in parentheses.

	Predicted docking CYP51 positions with propiconazole ligand (amino acids within 7Å)																						Global Distance Test with reference
Model/Position	**122**	**125**	**130**	**136**	**237**	**312**	**313**	**317**	**321**	**377**	**380**	**381**	**382**	**383**	**384**	**385**	**386**	**387**	**523**	**524**	**525**	**526**	
Amino acid	Y	L	F	Y	F	M	A	S	T	L	H	A	P	I	H	S	I	L	L	L	F	S	100%
C86 reference	5.40	4.73	6.27	6.28	6.65	5.43	2.91	1.27	3.37	3.13	4.80	5.03	2.48	1.85	3.85	4.85	7.04	5.74	>7Å	5.87	4.91	4.05	
Amino acid	Y	L	F	Y	F	M	A	S	T	L	H	A	P	I	H	S	I	L	L	L	F	S	85.61%
Bo_1	6.53	8.19	5.34	5.81	15.03	5.21	2.35	2.70	3.37	3.08	5.45	5.30	3.03	1.33	4.50	5.18	7.27	6.38	>7Å	8.21	4.90	4.40	
Amino acid	Y	L	F	Y	F	M	A	S	T	L	N	G	P	I	H	S	I	L	L	L	F	S	83.41%
CaM10_21 (Y463D)	6.24	8.29	5.64	5.95	7.64	4.95	2.55	3.03	3.32	3.51	5.56	3.89	2.31	1.40	4.15	5.26	7.03	6.09	>7Å	7.05	5.06	4.24	
Amino acid	Y	L	F	Y	F	M	G	S	T	L	H	G	P	I	H	S	I	L	L	L	F	S	82.02%
Z4_16 (G462A)	6.11	7.36	5.46	5.58	13.55	5.40	2.30	1.27	2.93	3.20	4.90	4.03	2.72	1.70	4.16	5.11	7.40	6.19	>7Å	9.08	4.92	3.81	
Amino acid	Y	L	F	Y	F	M	G	S	T	L	H	A	P	I	H	S	I	L	L	F	S	R	81.80%
M52_10 (ΔY461)	5.33	5.44	5.48	5.90	9.31	4.87	2.13	1.50	2.68	2.85	4.90	5.01	3.24	1.90	4.23	5.39	7.63	6.58	4.92	6.80	5.23	8.13	
Amino acid	Y	L	F	Y	F	M	G	S	T	L	H	A	P	I	H	S	I	L	L	L	F	S	80.42%
M52_22 (Y461N)	4.57	8.05	6.12	5.82	8.53	5.83	2.54	3.01	2.77	3.76	4.83	5.52	2.41	1.69	4.10	4.94	7.25	5.53	>7Å	8.41	7.86	4.24	
Amino acid	Y	L	F	F	F	M	G	S	T	L	H	A	P	I	H	S	I	L	L	L	F	S	76.95%
CaM10_6 (Y463D)	4.10	5.54	6.35	6.53	5.32	5.50	2.35	1.27	3.08	3.64	4.88	6.24	2.21	1.83	3.82	4.69	5.63	6.23	>7Å	5.03	4.25	3.79	

Table S8. Table with the information of the *Pseudocercospora fijiensis* CYP51 substitutions and *Pfcyp51* promoter sequences, including the number of palindromic sequences present, per isolates, DMI fungicides (difenoconazole, epoxiconazole and propiconazole) and country with their respective EC_{50} mean values. The online file will include the aligned sequence of the *Pfcyp51* gene and promoter of each sequenced isolate. (Excel file).

N	n	Isolate	TCGTACGA	T18	A19	Y58	I70	D71	V106	V116	Y136	K171	V260	I264	A313	H380	A381	R418G	A446	D460	Y461	G462	Y463	Difen.	Epoxi.	Propi.
COLOMBIA																										
1	1	Almendros_2	4	T18I					V106D		Y136F												Y463D	5.936	1.178	2.993
2	2	Almendros_3	4	T18I					V106D		Y136F												Y463D	0.887	0.537	0.831
3	3	Almendros_4	4	T18I					V106D		Y136F												Y463D	0.332	0.469	0.755
4	4	Bejuquillo_1	1	T18I	A19E				V106D															0.009	0.015	0.016
5	5	Bejuquillo_2	1	T18I					V106D															0.011	0.015	0.027
6	6	Bejuquillo_4	1	T18I					V106D															0.006	0.015	0.021
7	7	Bejuquillo_5	1	T18I					V106D															0.015	0.018	0.051
8	8	Bejuquillo_6	1	T18I					V106D															0.013	0.016	0.034
9	9	Bejuquillo_7	1	T18I					V106D															0.014	0.025	0.032
10	10	Bonita_2	1	T18I					V106D						A313G						Y461N			0.235	0.149	0.288
11	11	Caribe_1	6	T18I					V106D						A313G								Y463D	7.468	5.409	6.839
12	12	Caribe_2	6	T18I					V106D						A313G								Y463D	3.038	1.706	1.145
13	13	Caribe_3	4	T18I					V106D		Y136F												Y463D	5.694	2.5	3.181
14	14	C080910	1	T18I					V106D						A313G								Y463H	0.123	0.118	0.442
15	15	Esmeral._1	4	T18I					V106D		Y136F												Y463D	6.407	2.78	4.227
16	16	Esmeral._3	4	T18I					V106D		Y136F												Y463D	3.139	2.148	2.095
17	17	Espe._4	4	T18I					V106D		Y136F				A313G						Y461D			5.031	4.191	8.263
18	18	Estadero_1	4	T18I					V106D		Y136F												Y463D	>10.24	5.248	>10.26
19	19	Estadero_2	4	T18I					V106D		Y136F												Y463D	2.602	1.018	1.079
20	20	Estadero_3	4	T18I					V106D		Y136F												Y463D	2.769	0.535	2.227

N	n	Isolate	TCGTACGA	T10	A19	Y50	I70	D71	V106	V116	Y136	K171	V260	I264	A313	H380	A381	R419G	A446	D460	Y461	Q462	Y463	Difen.	Epoxi.	Propi.
21	21	Estadero_4	3	T18I					V106D						A313G								Y463H	0.94	0.569	0.953
22	22	Estadero_5	4	T18I					V106D		Y136F												Y463D	4.419	0.745	4.621
23	23	Frontera 2	4	T18I					V106D		Y136F												Y463D	2.746	0.801	2.878
24	24	Frontera 3	4	T18I					V106D		Y136F												Y463D	1.939	0.633	2.345
25	25	Frontera 5	4	T18I					V106D		Y136F												Y463D	1.678	1.111	3.91
26	26	Galvia_2	1	T18I					V106D						A313G						Y461N			0.372	0.194	0.694
27	27	Hon_3	4	T18I					V106D		Y136F				A313G						Y461D			7.074	3.168	8.139
28	28	Hon_4	4	T18I					V106D		Y136F												Y463D	5.125	0.676	3.502
29	29	Monte_3	4	T18I					V106D		Y136F												Y463D	4.183	0.927	2.22
30	30	Monte_4	4	T18I					V106D		Y136F												Y463D	4.909	1.716	3.569
31	31	Santillana 2	4	T18I					V106D		Y136F												Y463D	3.378	1.116	2.002
32	32	Santillana 3	4	T18I					V106D		Y136F												Y463D	1.853	0.328	1.805
33	33	Santillana 4	4	T18I					V106D		Y136F												Y463D	4.596	0.946	1.455
34	34	Trenza_3	1	T18I					V106D						A313G		A381G						Y463H	0.691	1.259	3.874
PHILIPPINES																										
41	7	B11_5	4	T18I					V106D						A313G				A446S	D460E	Y461N			5.487	4.545	4.853
42	8	B11_7	1						V106D						A313G				A446S	D460E	Y461N			0.713	0.324	1.087
43	9	B11_9	1						V106D						A313G				A446S	D460E	Y461N			1.468	0.757	1.163
44	10	B11_10	1						V106D						A313G				A446S	D460E	Y461N			1.564	2.1	3.031
45	11	B11_11	1						V106D						A313G				A446S	D460E	Y461N			0.508	0.235	0.667
46	12	B11_12	1						V106D						A313G				A446S	D460E	Y461N			0.56	0.346	0.833
47	13	B11_13	1						V106D						A313G				A446S	D460E	Y461N			0.364	0.266	1.138
48	14	Bo_1	1	T18I					V106D										A446S					0.006	0.011	0.017
49	15	M52_1	1						V106D						A313G				A446S	D460E	Y461N			0.328	0.177	0.776
50	16	M22_3	1	T18I				I70MI	D71E	V106D					A313G				A446S		Δ(Y461)			0.503	0.435	2.287

N	n	Isolate	TCGTACGA	T18	A19	Y58	I70	D71	V106	V116	Y136	K171	V260	I264	A313	H380	A381	R418G	A446	D460	Y461	G462	Y463	Difen.	Epoxi.	Propi.
51	17	MS2_4	1	T18I					V106D						A313G				A446S		Y461S			0.237	0.149	0.614
52	18	MS2_6	1						V106D						A313G				A446S	D460E	Y461N			0.168	0.194	1.029
53	19	MS2_7	4	T18I					V106D						A313G								Y463D	2.804	2.393	0.853
54	20	MS2_9	1	T18I					V106D			K77R			A313G				A446S		Y461D			0.533	0.336	0.67
55	21	MS2_10	1	T18I			I70M	D71E	V106D						A313G				A446S		Δ(Y461)			0.388	0.242	1.14
56	22	MS2_12	1						V106D						A313G				A446S	D460E	Y461N			0.521	0.841	3.905
57	23	MS2_14	1						V106D						A313G				A446S	D460E	Y461N			0.764	0.69	0.612
58	24	MS2_22	1						V106D						A313G				A446S	D460E	Y461N			1.866	0.807	1.345
59	25	MS2_23	1	T18I					V106D			K77R			A313G				A446S		Y461S			0.51	0.439	2.804
60	26	MS2_24	1						V106D			K77R			A313G				A446S	D460E	Y461N			0.22	0.127	1.226
61	27	MS2_25	1						V106D						A313G				A446S	D460E	Y461N			0.093	0.125	0.565
62	28	TS2_1	4	T18I					V106D						A313G				A446S	D460E	Y461N			5.528	1.849	2.264
63	29	TS2_2	4	T18I					V106D		Y136F				A313G								Y463D	10.035	2.486	8.791
64	30	TS2_4	4	T18I					V106D		Y136F				A313G								Y463D	>10.24	>10.24	>10.24
65	31	TS2_22	4	T18I					V106D		Y136F				A313G								Y463D	7.093	3.328	8.979
66	32	TS2_9	4	T18I					V106D		Y136F				A313G								Y463D	>10.24	>10.24	>10.24
67	33	U22_1	4	T18I					V106D						A313G								Y463D	1.306	1.365	1.709
68	34	U22_3	1	T18I					V106D			K77R			A313G				A446S		Y461D			0.781	0.423	0.54
ECUADOR																										
63	1	E_22	1	T18I					V106D										A446S					0.005	0.008	0.011
64	2	EC_1	1	T18I					V106D										A446S					0.005	0.008	0.015
65	3	EC_5	1	T18I	A19E				V106D										A446S					0.007	0.009	0.011
66	4	ENS_1	1	T18I					V106D										A446S					<0.004	0.04	0.03
67	5	ENS_52	1	T18I					V106D										A446S					0.006	0.008	0.013
68	6	ENV_9	1	T18I					V106D										A446S					<0.004	0.006	0.01
69	7	GNO_18	3	T18I					V106D						A313G						Y461D			1.376	1.132	2.823

N	n	Isolate	TCS1/ACS4	T18	A19	Y58	I70	D71	V106	V116	Y136	K171	V260	I264	A313	H380	A381	R410S	A446	D460	Y461	G462	Y463	Difen.	Epoxi.	Propi.
70	8	GNP_3	3	T18I					V106D						A313G								Y463H	2.709	1.589	5.351
71	9	GSB_17	1	T18I					V106D						A313G								Y463H	?	?	?
72	10	GCHA_4	1	T18I					V106D						A313G								Y463N	0.662	0.07	0.103
73	11	GCHS_7	1	T18I					V106D						A313G								Y463H	0.114	0.069	0.161
74	12	GCSB_13	1	T18I					V106D						A313G								Y463H	0.179	0.119	0.271
75	13	GS_4	1	T18I					V106D						A313G								Y463N	?	?	0.465
76	14	GS_10	1	T18I					V106D						A313G								Y463N	?	?	0.458
77	15	Gsa_13	1	T18I					V106D						A313G								Y463N	0.205	0.111	0.29
78	16	GSB_5	1	T18I					V106D						A313G								Y463N	0.232	0.144	0.261
79	17	GSB_7	3	T18I					V106D						A313G								Y463N	0.808	0.413	1.006
80	18	GSB_11	1	T18I					V106D						A313G								Y463N	0.05	0.05	0.192
81	19	GSN_1	1	T18I					V106D						A313G								Y463N	0.387	0.193	0.353
82	20	OCAL_5	1	T18I					V106D						A313G								Y463N	?	?	0.69
83	21	OCAL_6	1	T18I					V106D						A313G								Y463N	0.388	0.135	0.356
84	22	OCAL_11	1	T18I					V106D						A313G								Y463H	0.194	0.087	0.276
85	23	OCAL_12	1	T18I					V106D						A313G								Y463H	0.85	0.286	1.374
86	24	OMAL_9	1	T18I					V106D						A313G								Y463N	0.633	0.964	1.02
87	25	ONP_20	1	T18I					V106D						A313G								Y463D	?	?	1.99
88	26	ONS_1	1	T18I					V106D						A313G								Y463N	?	?	0.32
89	27	ONS_5	1	T18I					V106D						A313G								Y463N	?	?	3.16
90	28	OSSR_24	1	T18I					V106D						A313G								Y463N	?	?	2.08
91	29	OSSR_51	1	T18I					V106D						A313G								Y463H	0.323	0.541	0.945
92	30	RCSS_3	3	T18I					V106D						A313G								Y463N	1.501	0.921	2.942
93	31	RCSS_16	3	T18I					V106D						A313G								Y463N	2.324	0.942	2.761
94	32	RN_3	1	T18I					V106D						A313G								Y463H	?	?	0.271

N	n	Isolate	TCGTACGA	T18	A19	Y58	I70	D71	V106	V116	Y136	K171	V260	I264	A313	H380	A381	R418G	A446	D460	Y461	G462	Y463	Difen.	Epoxi.	Propi.
95	33	RN_5	1	T18I					V106D						A313G								Y463H	?	?	0.404
96	34	RS_13	1	T18I					V106D						A313G								Y463N	?	?	0.103
97	35	RSe6_6	1	T18I					V106D															0.086	0.02	0.066
98	36	RSSE_16	1	T18I					V106D						A313G								Y463N	0.575	0.626	1.026
99	37	RSSE_22	1	T18I					V106D						A313G								Y463N	0.259	0.141	0.527
100	38	RSSI_6	1	T18I					V106D						A313G								Y463N	0.269	0.159	0.527
101	39	SaR_2	1	T18I					V106D						A313G						Y461D			?	?	0.147
102	40	SaR_5	1	T18I					V106D						A313G								Y463N	?	?	0.251
COSTARICA																										
103	1	CaM1_2	6	T18I					V106D		Y136F				A313G								Y463D	4.088	2.859	5.668
104	2	CaM1_3	6	T18I					V106D		Y136F		V260L		A313G								Y463D	5.384	3.722	3.464
105	3	CaM1_4	6	T18I					V106D							H380N	A381G						Y463N	3.129	3.883	4.644
106	4	CaM1_5	6	T18I					V106D		Y136F		V260L		A313G								Y463D	13.054	5.176	6.191
107	5	CaM1_8	6	T18I					V106D							H380N	A381G						Y463N	2.788	4.44	1.931
108	6	CaM2_2	6	T18I					V106D		Y136F				A313G								Y463D	8.082	4.205	4.863
109	7	CaM2_3	6	T18I					V106D		Y136F				A313G								Y463D	4.977	5.094	4.13
110	8	CaM2_1	6	T18I					V106D		Y136F				A313G								Y463D	18.741	3.84	8.328
111	9	CaM2_3	6	T18I					V106D		Y136F						A381G						Y463D	3.202	1.016	2.783
112	10	CaM7_10	6	T18I					V106D		Y136F						A381G				Y461D			7.126	4.871	2.164
113	11	CaM7_19	6	T18I					V106D		Y136F				A313G		A381G				Y461D			6.042	4.41	5.069
114	12	CaM10_6	6	T18I					V106D		Y136F				A313G								Y463D	20.925	6.208	#####
115	13	CaM10_16	6	T18I					V106D		Y136F												Y463D	6.014	2.321	5.609
116	14	CaM10_21	6	T18I					V106D							H380N	A381G						Y463D	7.787	#####	6.269
117	15	SRM2_1	5	T18I					V106D						A313G								Y463D	2.658	1.651	2.801
118	16	SRM2_2	5	T18I					V106D						A313G								Y463D	1.599	1.051	1.323

N	n	Isolate	TCGTACGA	T18	A19	Y58	I70	D71	V106	V116	Y136	K171	V260	I264	A313	H380	A381	R418G	A446	D460	Y461	G462	Y463	Difen.	Epoxi.	Propi.
119	17	SPAD_3	5	T18I					V106D						A313G								Y463D	2.855	1.502	1.755
120	18	SPAB_1	5	T18I					V106D		Y136F				A313G								Y463I	7.91	4.448	5.388
121	19	SPMT_1	3	T18I					V106D		Y136F				A313G								Y463D	4.167	1.609	5.156
122	20	Zent1_2	5	T18I					V106D						A313G								Y463D	0.555	0.433	0.339
123	21	Cal_5	3	T18I					V106D		Y136F						A381G						Y463D	?	0.27	3.16
124	22	CaIL_16	6	T18I					V106D		Y136F												Y463D	5.613	1.255	2.776
125	23	CaIL_11	6	T18I					V106D		Y136F												Y463D	?	?	2.75
126	24	Cal10_13	6	T18I					V106D		Y136F												Y463D	5.604	3.385	3.971
127	25	Z4_7	1	T18I					V106D						A313G								Y463S	?	?	0.241
128	26	Z4_11	1	T18I					V106D						A313G								Y463H	?	?	0.171
129	27	Z4_14	1	T18I					V106D						A313G								Y463D	?	?	0.108
130	28	Z4_16	1	T18I					V106D								A381G					G462A		?	?	0.138
131	29	Z8_42	3	T18I					V106D		Y136F												Y463D	?	?	0.222
132	30	Z8_17	1	T18I					V106D						A313G								Y463S	?	?	0.114
133	31	Z8_18	3	T18I					V106D		Y136F												Y463D	?	?	0.162
134	32	ZTSC_77	1	T18I					V106D						A313G								Y463S	?	?	0.265
135	33	ZTSC_79	1	T18I					V106D															?	?	0.41
DOMINICAN REPUBLIC																										
136	1	A7	1	T18I					V106D						A313G								Y463N	0.16	0.05	0.271
137	2	A8	1	T18I					V106D						A313G								Y463H	0.24	0.09	0.364
138	3	A10	1	T18I					V106D								A381G							0.087	0.053	0.179
139	4	A12	1	T18I					V106D									R418G						0.007	0.012	0.016
140	5	A13	6	T18I					V106D						A313G								Y463D	2.17	0.929	1.112
141	6	A14	1	T18I					V106D						A313G								Y463H	0.291	0.207	0.291
142	7	A15	6	T18I					V106D						A313G								Y463D	1.837	1.165	1.569

N	n	Isolate	TCGTACGA	T18	A19	Y68	I70	D71	V106	V116	Y136	K171	V260	I264	A313	H380	A381	R418G	A446	D460	Y461	G462	Y463	Diflen.	Epoxi.	Propi.
143	8	Dom 001	1	T18I					V106D						A319G								Y463I	0.079	0.028	0.197
144	9	Dom 002	6	T18I					V106D						A319G								Y463D	1.049	0.963	0.866
145	10	Dom 003	6	T18I					V106D						A319G								Y463D	0.901	0.841	1.322
146	11	Dom 006	6	T18I					V106D						A319G								Y463D	0.518	0.653	1.037
147	12	Dom 007	6	T18I					V106D						A319G								Y463D	1.201	1.211	1.475
148	13	Dom 009	6	T18I					V106D						A319G								Y463D	0.893	0.687	1.133
149	14	Dom 014	6	T18I					V106D						A319G								Y463D	?	?	?
150	15	Dom 016	6	T18I					V106D						A319G								Y463D	?	?	?
151	16	Dom 029	6	T18I					V106D						A319G								Y463D	?	?	?
152	17	Dom 036	5	T18I					V106D		Y136F						A381G						Y463N	?	?	?
153	18	Dom 037	3	T18I					V106D		Y136F						A381G						Y463N	?	?	?
154	19	O1	6	T18I					V106D						A319G								Y463D	2.863	1.57	3.004
155	20	O2	5	T18I					V106D						A319G								Y463S	1.411	0.766	1.344
156	21	O3	6	T18I					V106D						A319G								Y463D	1.617	1.288	3.321
157	22	O4	6	T18I					V106D						A319G								Y463D	2.181	1.492	1.626
158	23	O5	6	T18I					V106D						A319G								Y463D	0.578	0.567	0.89
MARTINIQUE																										
159	1	Ma_26_2	1	T18I					V106D															0.043	0.025	0.015
160	2	Ma_26_16	1	T18I					V106D															0.029	0.029	0.02
161	3	Ma_26_18	1	T18I					V106D					I264T										0.072	0.035	0.027
162	4	Ma_26_30	1	T18I					V106D															0.005	0.008	0.008
163	5	Ma_26_32	1	T18I					V106D															0.005	0.005	0.013
GUADALUPE																										
164	1	GW_33	1	T18I					V106D															0.005	0.011	0.027
165	2	GW_35	1	T18I					V106D															0.012	0.012	0.026
166	3	GW_36	1	T18I					V106D															0.02	0.018	0.012

N	n	Isolate	TCGTACGA	T18	A19	Y68	I70	D71	V106	V116	Y136	K171	V260	I264	A313	H380	A381	R418G	A446	D460	Y461	G462	Y463	Difen.	Epoxi.	Propi.
CAMEROON																										
167	1	P2S_7	3						V106D						A313G								Y463D	?	?	?
168	2	P2S_9	2						V106D						A313G							G462D		0.107	0.107	0.338
169	3	P2S_14	3						V106D						A313G					D460V				1.665	1.048	1.795
170	4	P2S_18b	4						V106D						A313G					D460V				0.715	0.661	1.401
171	5	P2S_22b	1						V106D															0.005	0.007	0.011
172	6	P2S_29	3						V106D						A313G					D460V				1.598	0.729	1.883
173	7	P2S_45	4						V106D						A313G								Y463N	?	?	?
174	8	P2S_53	3						V106D						A313G					D460V				0.096	0.108	0.41
175	9	P2S_54	3						V106D						A313G					D460V				0.622	0.427	0.946
176	10	P2S_58b	3						V106D						A313G					D460V				0.096	0.108	0.413
177	11	P2S_63	1						V106D															?	?	?
178	12	P2S_72	3						V106D						A313G					D460V				1.917	1.155	1.388
179	13	P2S_76a	3						V106D						A313G					D460V				0.196	0.183	0.458
180	14	P2S_76b	3						V106D						A313G					D460V				?	?	?
181	15	P2S_84b	1						V106D															0.011	0.017	0.019
182	16	P2S_71b	3						V106D						A313G					D460V				0.199	0.149	0.394
183	17	P2S_16	1						V106D															0.01	0.015	0.042
184	18	P2S_18	3						V106D						A313G					D460V				0.715	0.661	1.401
185	19	P2S_22a	1						V106D															<0.004	0.004	0.006
186	20	P2S_59a	1						V106D						A313G								Y463D	0.55	0.131	0.743
187	21	P2S_84a	3						V106D						A313G					D460V				0.37	0.336	0.618
188	22	P2S_91	1						V106D						A313G							G462D		0.055	0.058	0.188
189	23	P2S_7	3						V106D						A313G					D460V				0.614	0.543	0.891
190	24	P2S_14	3						V106D						A313G					D460V				1.353	1.026	2.347
191	25	P2S_16	3						V106D						A313G					D460V				0.504	0.338	0.713
192	26	P2S_19	3						V106D						A313G					D460V				0.67	0.461	1.327

N	n	Isolate	TCGTACGA	T18	A19	Y58	I70	D71	V106	V116	Y136	K171	V260	I264	A313	H380	A381	R418G	A446	D460	Y461	G462	Y463	Difen.	Epoxi.	Propi.
193	27	P2S_20	4						V106D						A313G					D460V				0.687	0.921	1.245
194	28	P2S_24	4						V106D						A313G								Y463N	0.775	0.304	1.509
195	29	P2S_25	3						V106D						A313G					D460V				0.317	0.306	0.772
196	30	P2S_31	3						V106D						A313G					D460V				0.637	0.417	1.154
197	31	P2S_37	3						V106D						A313G					D460V				0.553	0.288	0.666
198	32	P2S_40	3						V106D						A313G					D460V				0.715	0.461	1.039
199	33	P2S_41	3						V106D						A313G								Y463N	1.12	0.599	1.653
200	34	P2S_42	3						V106D						A313G					D460V				0.537	0.589	1.204
201	35	P2S_44	3						V106D						A313G								Y463D	0.491	0.337	0.687
202	36	P2S_47	3						V106D						A313G					D460V				1.519	1.184	1.882
203	37	P2S_56	3						V106D						A313G					D460V				?	?	?
204	38	P2S_62	3						V106D						A313G								Y463D	0.602	0.299	0.72
205	39	P2S_64	3						V106D						A313G					D460V				0.393	1.354	1.992
206	40	P2S_68	3						V106D						A313G					D460V				0.248	0.194	0.657
207	41	P2S_78	3						V106D						A313G					D460V				1.094	0.743	1.464
208	42	P2S_79	3						V106D						A313G					D460V				0.797	0.627	1.9
209	43	P2S_81	3						V106D						A313G					D460V				0.325	0.297	0.532
210	44	P2S_X	3						V106D						A313G					D460V				0.567	0.337	0.789
211	45	P4S_1	3						V106D						A313G					D460V				2.041	1.623	2.194
212	46	P4S_5	3						V106D						A313G					D460V				1.011	1.223	1.737
213	47	P4S_7a	4						V106D						A313G								Y463N	1.19	0.614	1.566
214	48	P4S_7b	3						V106D						A313G					D460V				1.487	1.055	1.814
215	49	P4S_13	3						V106D						A313G					D460V				1.141	0.975	0.809
216	50	P4S_16	3						V106D						A313G					D460V				0.833	0.843	1.144
217	51	P4S_19	4						V106D						A313G					D460V				0.564	0.48	1.38
218	52	P4S_22	3						V106D						A313G					D460V			Y463N	1.405	1.1	1.764
219	53	P4S_24	3						V106D						A313G					D460V				0.542	0.531	1.501

N	n	Isolate	TCGTACGA	T18	A19	Y58	I70	D71	V106	V116	Y136	K171	V260	I264	A313	H380	A381	R418G	A446	D460	Y461	G462	Y463	Difen.	Epoxi.	Propi.
220	54	R4S_28	3						V106D						A313G					D460V				0.48	0.427	1.047
221	55	R4S_29	2						V106D						A313G							G462D		?	?	?
222	56	R4S_33	3						V106D						A313G					D460V				0.581	0.86	1.286
223	57	R4S_38	3						V106D						A313G					D460V				0.454	0.345	0.542
224	58	R4S_42	3						V106D						A313G					D460V				0.549	0.463	1.348
225	59	R4S_47	1						V106D						A313G								Y463D	0.231	0.097	0.382
226	60	R4S_51	3						V106D						A313G					D460V				0.574	0.332	1.111
227	61	R4S_53	3						V106D						A313G					D460V				0.349	0.403	0.656
228	62	R4S_58	4						V106D						A313G								Y463D	0.576	0.536	1.132
229	63	R4S_60a	3						V106D						A313G					D460V				0.469	0.463	1.094
230	64	R4S_60b	4						V106D						A313G					D460V				0.562	0.603	1.081
231	65	R4S_64	4						V106D		Y136F				A313G							G462D		6.663	3.934	7.816
232	66	R4S_65	3						V106D						A313G					D460V				1.25	1.297	1.356
233	67	R4S_72	3						V106D						A313G					D460V				0.672	0.52	1.358
234	68	R4S_78	3						V106D						A313G					D460V				1.068	0.464	1.734
235	69	R4S_81	3						V106D						A313G					D460V				1.264	0.706	1.778
236	70	X02_04	1						V106D															0.005	0.008	0.011
237	71	X03_02	1						V106D															0.005	0.006	0.009
238	72	X04_02	1						V106D															0.004	0.007	0.012
239	73	X04_05	1						V106D															0.005	0.01	0.014
240	74	X05_03	1						V106D															0.007	0.009	0.013
241	75	X05_05	1						V106D															0.006	0.007	0.016
242	76	X07_02	1						V106D															0.006	0.011	0.016
243	77	X08_01	1						V106D															0.005	0.015	0.015
244	78	X08_02	1						V106D															0.005	0.009	0.014
245	79	X13_03	1						V106D															0.005	0.01	0.012
246	80	X14_03	1						V106D															0.005	0.009	0.016

N	n	Isolate	TCGTACGA	T18	A19	Y58	I70	D71	V106	V116	Y136	K171	V260	I264	A313	H380	A381	R418G	A446	D460	Y461	G462	Y463	Difen.	Epoxi.	Propi.
247	81	X14_04	1						V106D															0.005	0.009	0.012
248	82	X14_05	1						V106D															0.007	0.025	0.028
249	83	X16_01	1						V106D															0.006	0.013	0.017
250	84	X16_03	1						V106D															0.01	0.033	0.052
251	85	X18_05	1						V106D															0.007	0.017	0.02
252	86	X18_07	1						V106D															0.007	0.01	0.021
253	87	X18_08	1						V106D															0.006	0.01	0.017
254	88	X18_10	1						V106D															0.005	0.008	0.01
255	89	X19_01	1						V106D															0.006	0.011	0.016
256	90	X19_03	1						V106D															0.005	0.005	0.005
257	91	X23_02	1						V106D															0.005	0.007	0.013
258	92	X23_03	1						V106D															0.007	0.018	0.023
259	93	X24_02	1						V106D															0.006	0.01	0.014
260	94	X26_07	1						V106D															0.007	0.011	0.017
INDIVIDUAL SENSITIVE ISOLATES																										
261	1	X845 (Indonesia)	1	T18I		Y58F			V106D															?	?	0.079
262	2	X846 (Philippines)	1	T18I					V106D	V116L														?	?	0.055
263	3	X847 (Taiwan)	1						V106D			K171R							A446S					?	?	0.034
264	4	X849 (Burundi)	1						V106D															?	?	0.053
265	5	X851 (Gabon)	1						V106D															?	?	0.06
266	6	C_86*	1																					0.004	0.007	0.01

*Reference Isolate CIRAD86, Isolates in blue were used for *PfCYP51* in silico modelling

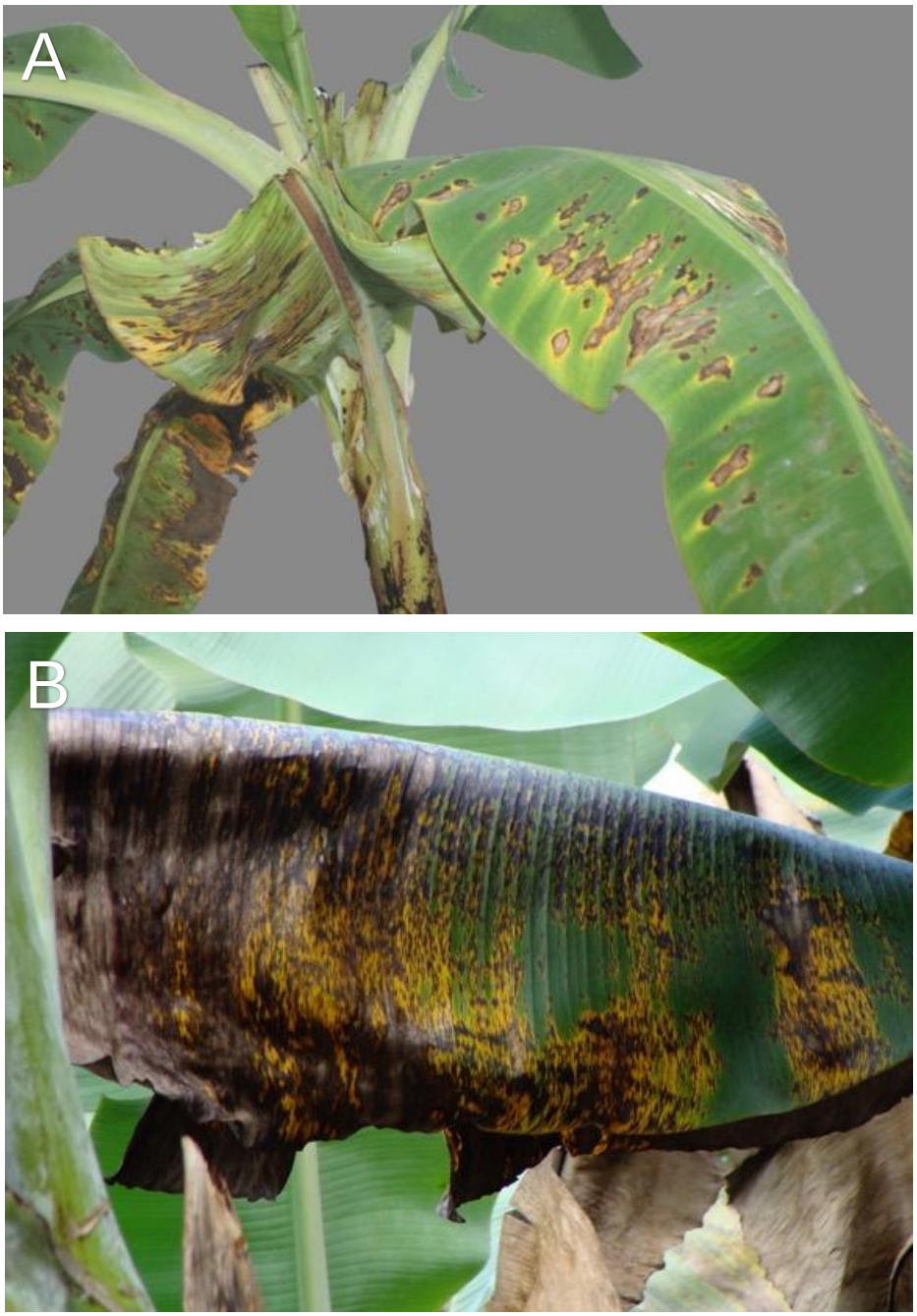


Figure S1. A. Banana plant infected with *Pseudocercospora. fijiensis* in a greenhouse experiment. The plant shows the typical symptom of the disease, elliptical necrotic lesions with water-soaked border and a chlorotic yellow halo**.** B. Symptoms of naturally infected banana plants in the field.

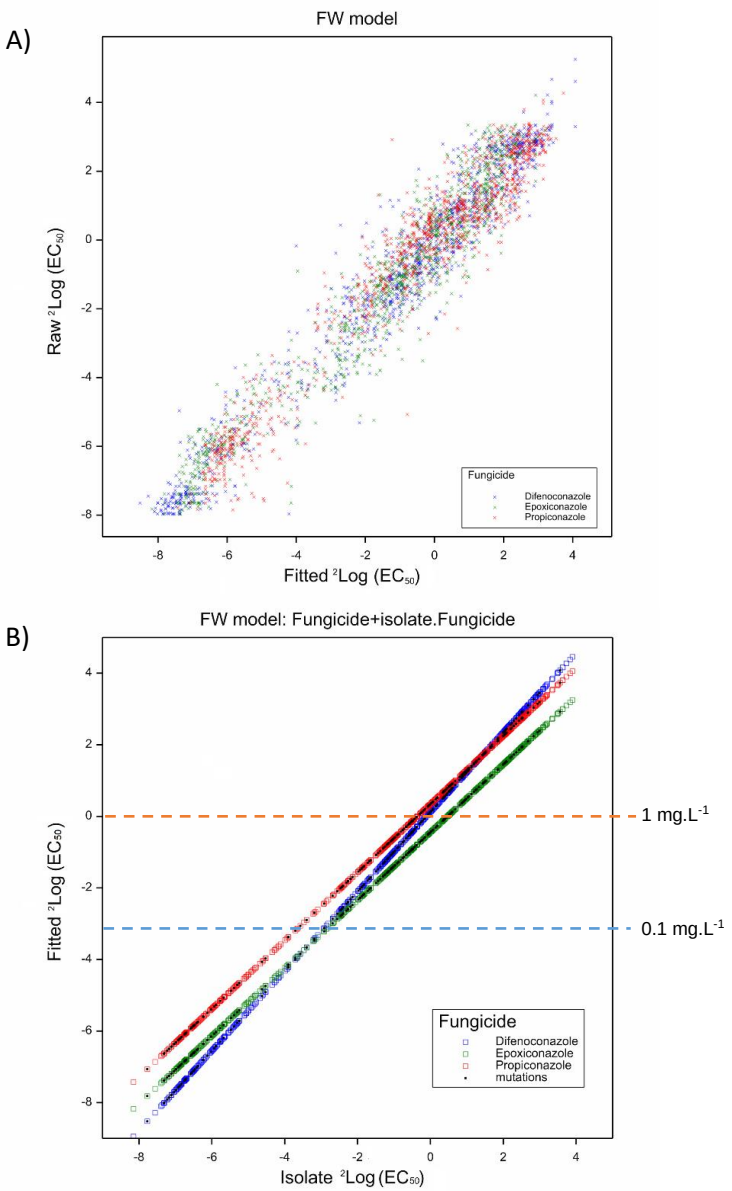


Figure S2. Plots of the Finlay-Wilkinson model (FW) describing the interaction between the three fungicides (model: y_{ijk} = Fungicide$_i$ + b_j x Isolate$_j$ + ε_{ijk} or y_{ijk} = Isolate$_i$ + b_j x Fungicide$_j$ + ε_{ijk}.) in 592 isolates of *Pseudocercospora fijiensis*. A) Individual strains ^{2}Log EC_{50} raw data (3 repeats) of the three DMI. B) Fitted ^{2}Log EC_{50} mean data. Sensitive and resistant threshold are show in blue and red dashes lines, respectively. Indicated in black dots are the isolates of which the *Pfcyp51* gene is sequenced. General difference between fungicides exist where isolates reacts proportionally by the EC_{50}. Nonetheless the main effects of isolate still describe nearly 92% of the variation in EC_{50} found.

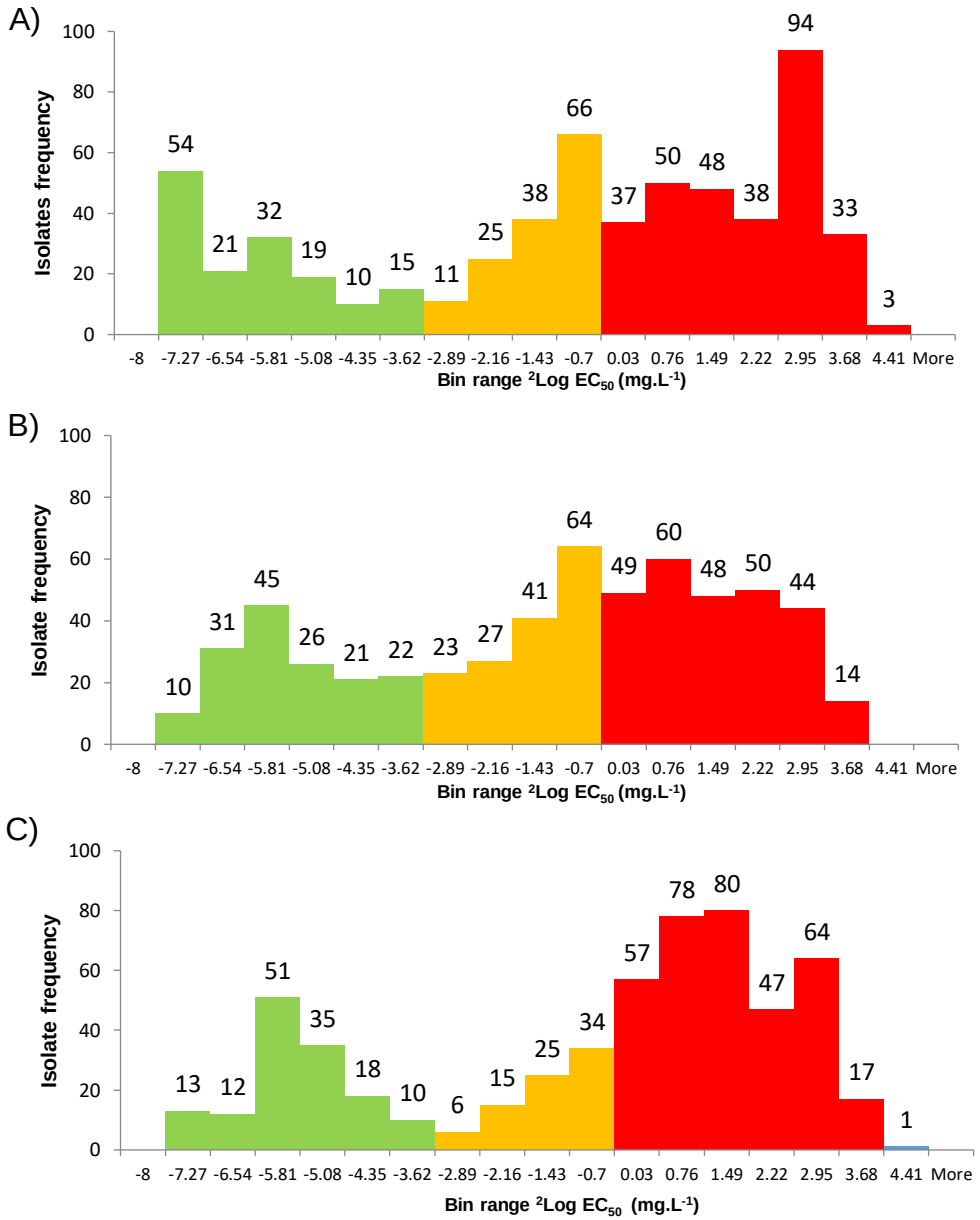


Figure S3. Plot of the ^{2}Log mean EC_{50} values distribution of the frequency of all *Pseudocercospora fijiensis* isolates and their interaction with each DMI fungicide: A) difenoconazole, B) epoxiconazole and C) propiconazole. The thresholds grouping criteria is coloured green for sensitivity, yellow for tolerance and red for resistance.

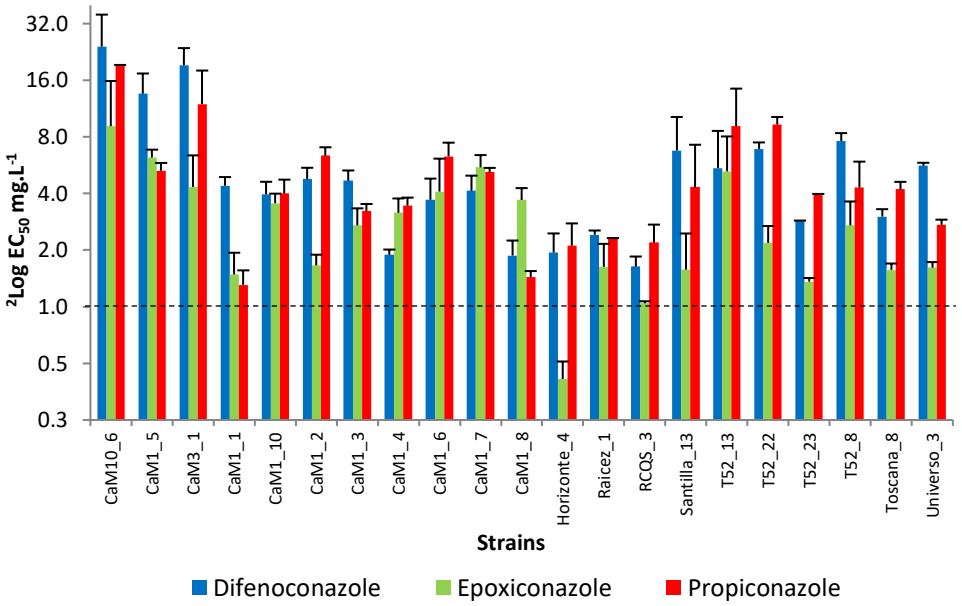


Figure S4. Calculated EC_{50} means of the tested *Pseudocercospora fijiensis* isolates with fungicide concentration up to 40.96 $mg.L^{-1}$. The dashed line represents the threshold value (1 $mg.L^{-1}$) for DMI resistant isolates.

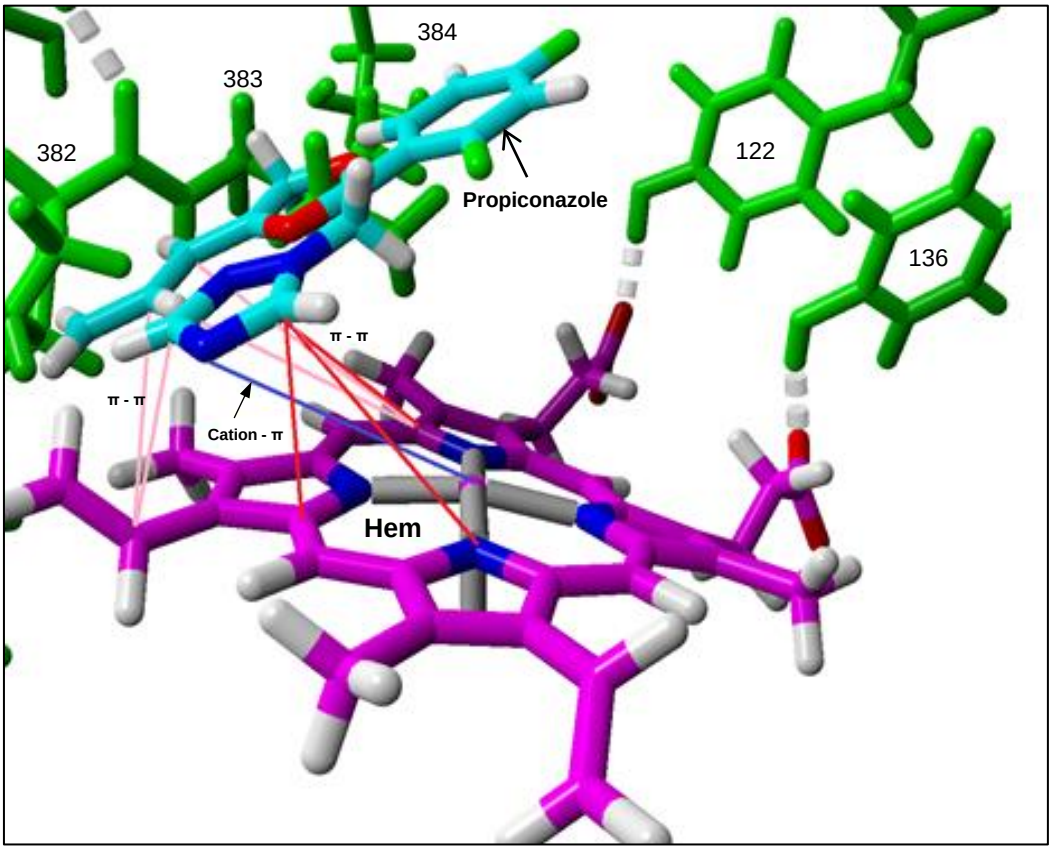


Figure S5. Predicted interaction of propiconazole in the binding site of *Pseudocercospora fijiensis* CYP51. Amino acid residues in the active site are shown in green. The heme group's carbon atoms are depicted in magenta and the propiconazole carbon atoms are shown in cyan. Hydrogen atoms are coloured in light grey, oxygen in red and nitrogen in blue. The iron atom is also depicted in magenta in the heme group. Interaction forces are shown in blue (cation-π), pink or red (π - π) lines.

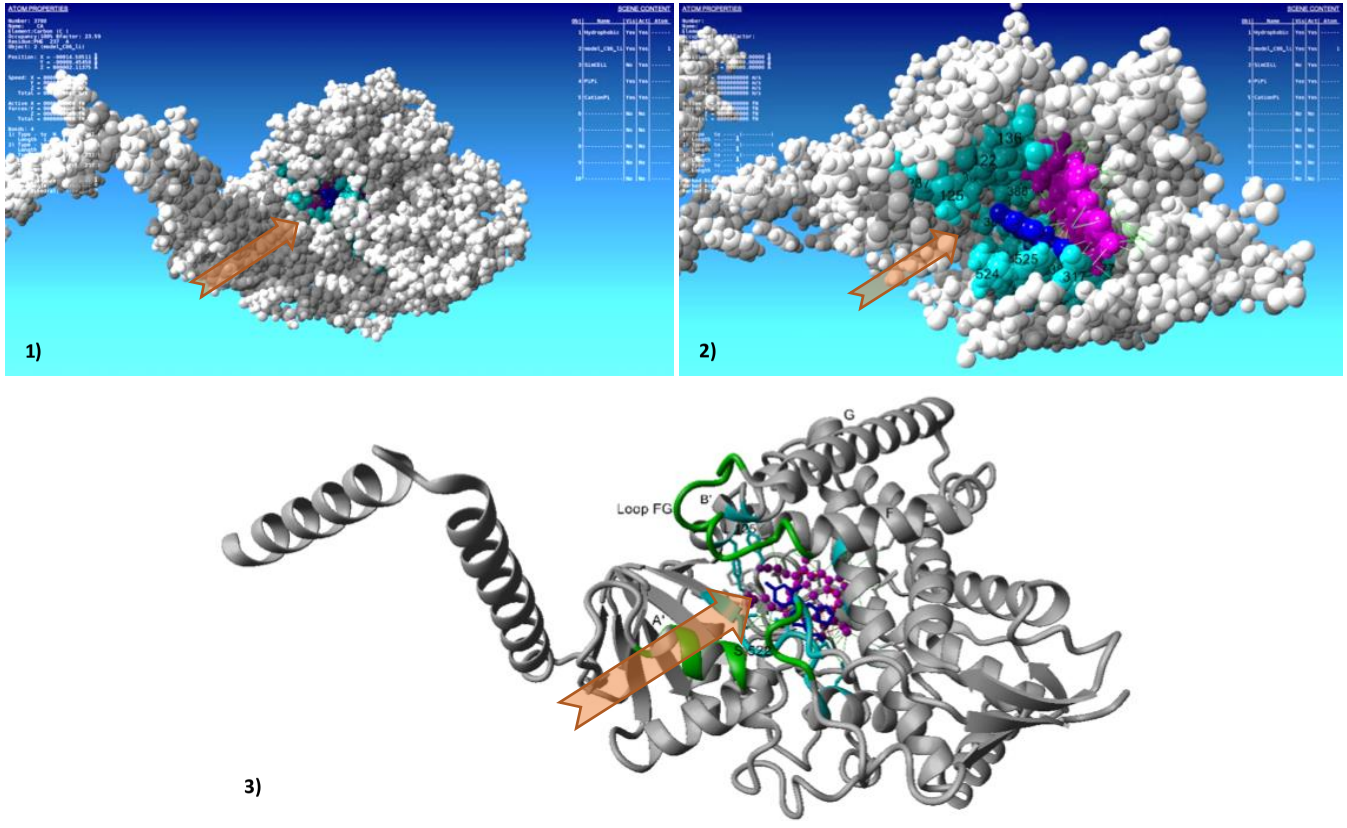
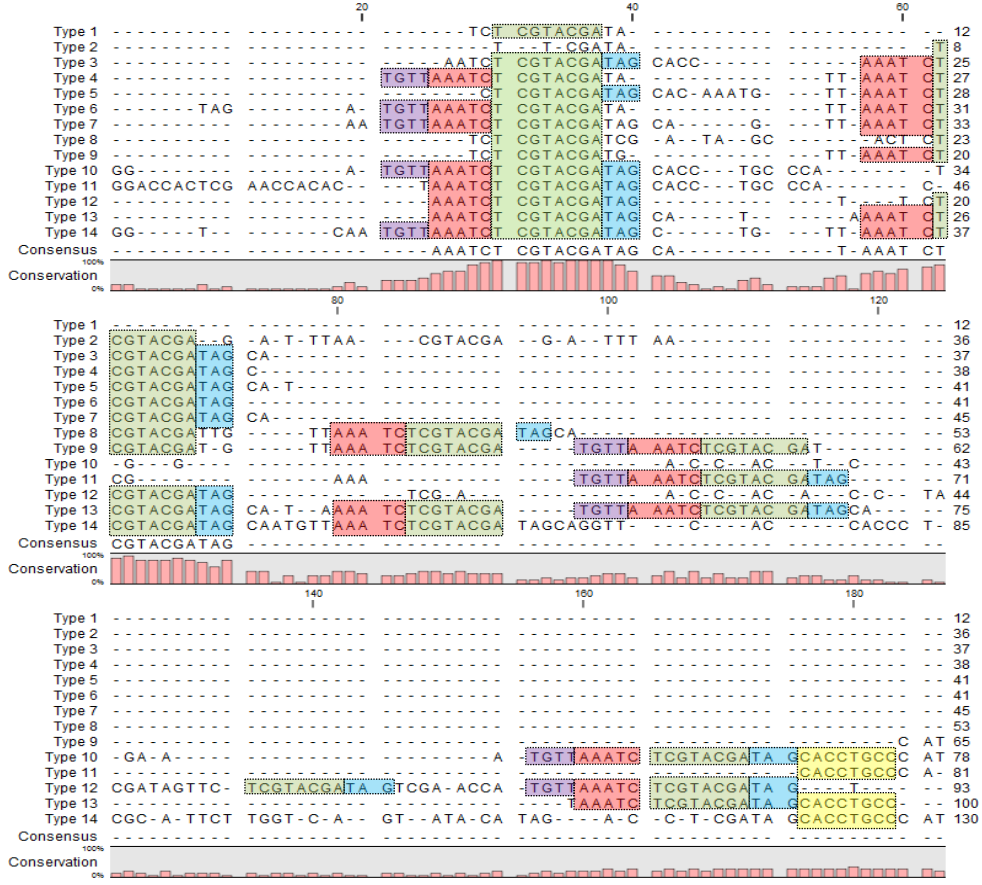


Figure S6. 1) Ball representation of reference *Pseudocercospora fijiensis* PfCYP51 where the channel leading to the active site is indicated (arrow). Amino acid (aa) residues located around the active cites are depicted in cyan. 2) Lateral dissection view of 1) where the aa that form the active site can be seen. The heme group is shown in magenta, propiconazole in blue and aa's closer than 7Å are shown in cyan (some aa's have been removed for a better look. 3) Ribbon and ligand representation of reference PfCYP51 showing 1) and 2) features. The structures surrounding the entrance of the channel are depicted in green. The red arrows indicate the entrance of the channel that leads to the active site.



Insertion type	bps	Strains	Country
Type 1	12	2	Cameroon
Type 2	36	2	Ecuador
Type 3	37	2	Ecuador
Type 4	38	49	Cameroon
Type 5	41	1	Costa Rica
Type 6	41	7	Cameroon
Type 7	45	1	Cameroon
Type 8	53	20	Colombia
Type 9	65	2	Costa Rica
		2	Dominican Republic
Type 10	78	1	Colombia
Type 11	82	1	Dominican Republic
		1	Costa Rica
Type 12	93	4	Costa Rica
Type 13	100	13	Dominican Republic
		17	Costa Rica
		2	Colombia
Type 14	130	8	Philippines

Figure S7. Alignment of the insertion variations identified in the promoter of the *Pseudocercospora fijiensis Pfcyp51*gene. Common sequences among different types of insertions are marked in boxes of different colours. The palindrome sequence "TCGTACGA" is marked in green. Different numbers to the right of the alignment represents the different base pairs length for each type.

Chapter 4

A new resistance mechanism to DMI fungicides in the fungal banana black Sigatoka pathogen *Pseudocercospora fijiensis* is driven by increased expression of *Pfcyp51* through multiple promoter repeats

Caucasella Diaz-Trujillo[†], Pablo Chong[†], Viviane Cordovez, Mauricio Guzmán, Pierre J.G.M. De Wit, Ioannis Stergiopoulos, Harold J. G. Meijer, Rafael E. Arango Isaza, Gabriel Scalliet, Helge Sierotzki, Esther Lilia Peralta and Gerrit H. J. Kema.

Summary

Black Sigatoka is one of the most important disease in bananas and plantains and the most relevant economically. Black Sigatoka is caused by the dothideomycete fungus *Pseudocercospora fijiensis,* previously known as *Mycosphaerella fijiensis.* Disease control is mainly obtained through the application of fungicides, including the lanosterol demethylation-inhibitors (DMIs). The continued use of DMI has triggered the appearance of novel genotypes, displaying reduced sensitivity to this class of fungicides. So far the phenotype of these isolates was found to be linked to the presence of non-synonymous point mutations in the target gene encoding the lanosterol 14α-demethylase enzyme (*Pfcyp51*). In this study, we identify a 19 base pairs (bp) repeat element in the promoter region (103 bp upstream the coding region) of the *Pfcyp51*gene, whose copy number correlates positively with increased resistance to DMIs. A PCR-based assay was developed to characterize four field populations of *P. fijiensis* in Costa Rica for the presence and copy numbers of repeated elements within the *Pfcyp51* promoter. Additionally, functional analyses - including promoter swapping - showed that the presence of the repeat element proportionally upregulates *Pfcyp51* expression which consequently decreases sensitivity to the DMIs in vivo. This study provides important information on the genetic mechanisms that confer reduced sensitivity to azole fungicides and might offer a tool for optimizing the use of azoles in disease management of black Sigatoka.

Introduction

Black Sigatoka, caused by the ascomycete *Pseudocercospora fijiensis* (Morelet) Deighton (1976), (synonym, *Mycosphaerella fijiensis* Morelet (1969)), is one of the most devastating and economically significant diseases for export bananas and plantains. Disease management of black Sigatoka is mainly based on the application of fungicides, in which

single-sites plays an important role. However, the high level of sexual reproduction of this fungus favours the generation and maintenance of highly diverse populations with a broad base-line sensitivity towards fungicides (Arango Isaza et al., 2016; Conde-Ferráez et al., 2007; Hayden and Carlier, 2003; Rivas et al., 2004; Romero and Sutton, 1997). As a result, fungicide resistance develops frequently and spreads rapidly, particularly when pathogen populations are under strong selection pressure (Arango *et al.* 2016; Ware *et al.* 2006). This situation has contributed to a dramatic increase in the number of fungicide applications, which can tally up to over 50 applications (12 azoles applications) per year in some banana export countries (Chong *et al.* 2016b; FRAC 2010; Lapeyre *et al.* 2010a; Martínez-Bolaños *et al.* 2012). This can dramatically increase production costs by as high as 30% (Marín et al., 2003) and additionally poses a threat to occupational health and the environment. It is thus imperative to understand the mechanisms by which resistance towards DMIs develops in order to enable adequate long-term disease management strategies with optimized chemical input.

Azoles fungicides have been used against black Sigatoka as early as 1987, but became widely used since 1991 when propiconazole, one of the currently prominent lanosterol 14α-demethylation inhibitors (DMIs), was introduce in the market (Chong *et al.* 2016a; Romero & Sutton 1997) (Chong et al., 2016a; Romero and Sutton, 1997). Currently, several DMI fungicides, such difenoconazole, bitertanol, and epoxiconazole are commonly used in spray programs (Chong et al., 2016a). DMI fungicides act as inhibitors of the CYP51 enzyme involved in the 14α-demethylation of the ergosterol precursor eburicol (24-methylene-24, 25-dihydrolanosterol). Ergosterol regulates cellular membranes fluidity and permeability, and is essential for cell viability (Lepesheva and Waterman, 2011). Resistance or reduced sensitivity for most single-site fungicides developed rapidly in *P. fijiensis* after introduction of strobilurins, benzimidazoles, and DMI for disease control in banana production (Arango et al., 2016; Amil et al., 2007; Cañas-Gutiérrez et al., 2009, 2006; Romero and Sutton, 1997).

Previous studies on *P. fijiensis* revealed the correlation between resistance to propiconazole and point mutations in the *Pfcyp51* gene, which caused amino acid (aa) substitutions surrounding the Substrate Recognition Site (SRS) at positions Y136, A313, Y461 and Y463 (Cañas-Gutiérrez et al., 2009; Chong et al., 2016b). Prior to this work, aa substitutions were the only described mechanisms for shifting sensitivity to azoles in *P. fijiensis*. Here, we report the presence and analysis of a repetitive element in the promoter region of *Pfcyp51* gene from *P. fijiensis* field strains that are resistant to propiconazole. Specifically, we have studied the presence and copy number of these elements in 239 field isolates that were collected in Costa Rican banana plantations with and without fungicide applications, and compared them with control isolates originating from Ecuador, Asia and Africa. This comparison enabled us to establish positive correlation between the presence and copy number of the elements in the *Pfcyp51* promoter, on one hand, and its overexpression and reduced fungicide sensitivity, on the other. The influence of promoter inserts, on increased target expression and reduced azole sensitivity was experimentally corroborated by using promoter swaps between propiconazole, difenoconazole and epoxiconazole sensitive and resistant *P. fijiensis* strains. These promoter inserts upstream of the *Pfcyp51* gene represent an additional resistance mechanism in *P. fijiensis.*

Materials and methods

Pseudocercospora fijiensis strains

A set of 25 monoascosporic *P. fijiensis* strains from Africa, Asia and Latin America, was used for fungicide sensitivity assays. Eight of the Latin-American strains were collected in Ecuador and 11 strains in Costa Rica. The larger set of Costa Rican strains was from four different banana plantations: Cartagena (Ca), Zent (Z), San Pablo (SP) and San Carlos (ZTSC)

(Arango et al., 2016). The former three are frequently sprayed with fungicides, whereas the San Carlos is a plantain growing area with low *P. fijiensis* incidence, hence fungicides are not required for disease control. We consider the *P. fijiensis* population from this area as a wt population. Strains were obtained from CORBANA (Costa Rica), CIBE-ESPOL (Ecuador) and CBS-KNAW Fungal Biodiversity Centre (Africa and Asia).

Determination of *in vitro* sensitivity to azole fungicides

The fungicides propiconazole and difenoconazole were provided by Syngenta Crop Protection AG, Basel, Switzerland. Epoxiconazole was obtained from Sigma (Sigma Aldrich, Missouri, USA). All compounds were technical grade quality and were kept in 100x stock solutions, either in methanol or DMSO. When applied to the culture medium the final concentration of the solvents was <1% (v/v). For the initial *in vitro* sensitivity assays the final concentrations tested for propiconazole were 10, 5.62, 3.16, 1.78, 1.0, 0.56, and 0.31 $mg \cdot L^{-1}$. Subsequently, to evaluate sensitive strains more accurately, lower concentrations of fungicides were included in the assays (10.24, 2.56, 0.64, 0.16, 0.04, 0.016, 0.004, 0 $mg \cdot L^{-1}$) and exploited to evaluate the performance of *P. fijiensis* transformants in the presence of propiconazole, difenoconazole and epoxiconazole.

Fungicide sensitivity of each strain was determined by calculating the 50% inhibitory concentration (EC_{50}). Quantitative analysis of fungal growth, was determined by the 96 -well microtiter plate dilution assay (Peláez *et al.* 2006) with some modifications. Fifty microliters of a $1x10^5$ mycelial parts/mL solution from each strain were inoculated in 200 µl potato dextrose broth medium per well of a 96-well polystyrene, flat bottom, transparent, plate (Corning, USA; cat. # 3370). Plates were incubated at 25 ºC in an incubator (Elbanton, Kerkdriel, Netherlands) for seven days before mycelial growth was measured. Each

concentration was tested in duplicate per strain, and per plate four blank controls were present. Individual plates were considered as one biological replicate, and tests were performed in triplicate. Absorbance was initially measured at 620 nm in a TECAN A5082 plate reader (Männedorf, Switzerland), but due to the variation of mycelial colours over the strains as well as the different colony morphologies, we eventually monitored growth at an absorbance of 690 nm in an Infinite® M200 PRO reader (TECAN, Männedorf, Switzerland), which enabled measuring higher sensitivities. The read design per well was settled at room temperature, leaving a border of 1,000 μm, a bandwidth of 9 μm, circle-filled reads of 25 read points (5x5), and each read point was measured 5 times. Read averages were plotted against dpi and compared with the other strains and controls. The fungicide sensitivity of transformants and control strains was determined by the aforementioned 96-well polystyrene plates. Sealed plates were maintained at 27 ºC in an incubator (Elbanton, Kerkdriel, Netherlands) in darkness and fungal growth was evaluated 10 days post inoculation (dpi). Plates were evaluated at 690 nm, while covered to reduce contamination.

Pfcyp51 **gene and promoter amplification and sequencing**

To amplify the *Pfcyp51* gene and the promoter region, specific primers located at the first repeat element and 22 bp upstream of the open reading frame (ORF) were used: *CYP51_Pfijien_F1* (5'-AAGGTCATATCGCAGG-3') and *CYP51_Pfijien_R1* (5'-GAATGTTATCGTGTGACA-3'). A basic PCR mix was prepared and the PCR program consisted of 5 min. of denaturation at 94 °C followed by 34 cycles of 30 sec. at 94 °C, 30 sec. of annealing at 55 °C and 90 sec. of extension at 68 °C. An additional extension step of 7 min. at 72 °C was performed at the end. DNA sequencing of the gene was performed at Macrogen (Seoul, Korea) and by the Genomics facility of Wageningen University and Research Centre (WUR), directly using the PCR products. In order to obtain the entire

sequence of the gene and the promoter region four primers were used in the sequencing reactions: *CYP51_Pfijien_F2* (5'-ACAGAAACATCACCTCC-3'), *CYP51_Pfijien_F3* (5'-ATTGCTTCACTTTCATCC-3'), *CYP51_Pfijien_F4* (5'-CTCTACCACGATCTCGAC-3') and *CYP51_Pfijien_R2* (5'-GATATGGATATAGTTGTC-3'). The obtained sequences were assembled in contigs per strain using CLC DNA Workbench software (CLC bio, Aarhus, Denmark) and the ORF was translated to aa and the protein sequences were aligned using the ClustalW plug in. The sequence alignments allowed the identification of mutations.

***Pfcyp51* gene expression analysis**

Extraction of total RNA was carried out with mycelia of *P. fijiensis* isolates grown for 10 days in liquid PDB using the Qiagen RNA extraction plus mini kit (QIAGEN Inc., Valencia, USA). The integrity of the RNA was checked using agarose gel electrophoresis and the concentration was determined by measuring absorbance at 260 nm in a nanodrop spectrophotometer (Thermo scientific, Wilmington, USA). Expression analysis was performed by quantitative real time -PCR (qRT-PCR) using primers *QRTCYP-forward*: (5'-CGCCAGTATTCGGCACAGATGTCG-3') and *QRTCYP-reverse:* (5'-TAACGTAGGACTGGAGGGCGGA-3'), which amplify a fragment of 89 bp of the *Pfcyp51* gene and primers *QRTACT-forward*: (5'-TCCGTCCTTGGTCTCGAATCTGGT-3') and *QRTACT-reverse*: (5'-TGCATACGGTCGGAGATACCTGGA-3'), which amplify a fragment 146 bp of the *P. fijiensis* actin gene that was used to normalize the expression. Quantitative RT-PCR reactions were performed using 20 ng of total RNA per strain in an Applied Biosystems ABI 7500 thermocycler (Waltham, USA) using the Applied Biosystems Power SYBR® Green RNA-to-CT™ 1-Step Kit, according to the manufactures instructions. The delta-delta Ct method was used - with the actin gene as the endogenous control - to determine the level of *Pfcyp51* gene expression (Livak & Schmittgen 2001).

Analysis of promoter repeats of *Pfcyp51* gene in four Costa Rican *P. fijiensis* populations

Genomic DNA (gDNA) of 225 *P. fijiensis* isolates from the four Costa Rican populations was analysed; 82 from the Cartagena population, 43 from the San Pablo population, 84 from the Zent population, and 16 from the San Carlos wt population. PCR fragments were amplified from gDNA using the specific primer pair, *P._fijiensis*_repeats_F (5'-TCTCGTACGATAGCACCTGCCCA-3') and *P._fijiensis*_repeats_R (5'-TGTTGGTGTAGGGGGTTAGGCCA-3') that was designed to amplify the promoter region of *Pfcyp51*. PCR conditions comprised 2 min. at 95 °C, 30 cycles of 30 sec. denaturation at 95 °C, 30 sec. of annealing at 68 °C, and 2 min. of extension at 72 °C with an additional extension step of 10 min. at 72 °C at the end of the reaction. PCR products were visualized and evaluated on a 1% agarose gels and eleven isolates were selected for sequencing and subsequent analysis of promoter and coding sequences. Different repeated elements were aligned and a weblogo consensus sequence was generated (Crooks *et al.* 2004) to graph nucleotide conservation within the elements.

Promoter swapping

We performed a promoter swapping experiment to test the effect of promoter repeats on *Pfcyp51* expression and henceforward on sensitivity to several azole fungicides. The *Pfcyp51* donor promoter for homologous recombination was obtained from the resistant strain Ca5_16. The recombination construct pPROM_CYP51_Ca5_16 comprised an upstream 2,024 bp fragment (the *PfCyp51* gene has an antisense position in the genome), obtained by using primers 5-CYP-Prom Fwd (5´-GGGGACAACTTTGTATAGAAAAGTTGAGGATATCAAGCACGCAC-3´) and Rev (5´-GGGGACTGCTTTTTTGTACAAACTTGGAAGAGAAACGGACTCCA-3´), which was cloned in front of a cassette with the hygromycin (*hph*) resistance gene and the green

fluorescent protein (*gfp*) gene, followed by the upstream region of 1,737 bp obtained with primers 3-CYP-Prom Fwd (5´- GGGGACAGCTTTCTTGTACAAAGTGGGAATGAGCATTTGAGAGC-3´) and Rev (5´-GGGGACAACTTTGTATAATAAAGTTAATACTAGCGGAGGTTCG-3´), containing the promoter region of strain Ca5_16, which has six promoter repeats. Transformations were performed by *Agrobacterium tumefaciens* mediated transformation (Díaz-Trujillo *et al.* 2016b) using the sensitive *P. fijiensis* strain E22, with a single repeat element and no mutations in the coding region. The promoter length of 250 GFP labelled transformants was compared with the promoter length of the resistant donor Ca5_16 and the sensitive recipient E22 strains. Transformants with a Ca5_16 sized promoter are considered to be homologous recombinants, hence promoter swapped transformants, which were subsequently analysed for the integration site using PCR of a 2,629 bp amplicon using primers PROM-HR-3´ Fwd (5´-TGAGCATTTGAGAGC-3´) and Rev (5´-TTATGATCGCCTCCAAGC-3´) located in the cassette and the *Pfcyp51* ORF, respectively.

Results

In vitro sensitivity to propiconazole

The *P. fijiensis* isolates that were tested for sensitivity to the azole fungicides were classified in three groups; strains with (1) EC_{50} values of ≤0.10 mg.L^{-1} were marked sensitive; (2) EC_{50} values between 0.10 to 0.90 mg.L^{-1} were consider tolerant and (3) those with EC_{50} values ≥1.0 mg.L^{-1} were consider resistant (Figure 1 and Table 1). Among the 25 isolates tested for sensitivity to propiconazole, 7 were sensitive, 14 moderately resistant and four were resistant. Clear cross-resistance was observed, since all isolates showed similar EC_{50} values (data not shown). In general, strains coming from banana plantations in Costa Rica and Ecuador displayed higher EC_{50} values compared to strains coming from Africa or Asia. Also

strains originating from frequently (>50/year) sprayed plantations (Chong et al., 2016a; De Lapeyre De Bellaire et al., 2010), such as Cartagena, showed significantly reduced sensitivities to the fungicides, contrary to strains coming from regions not subjected to fungicide applications (Figure 1, Table 1).

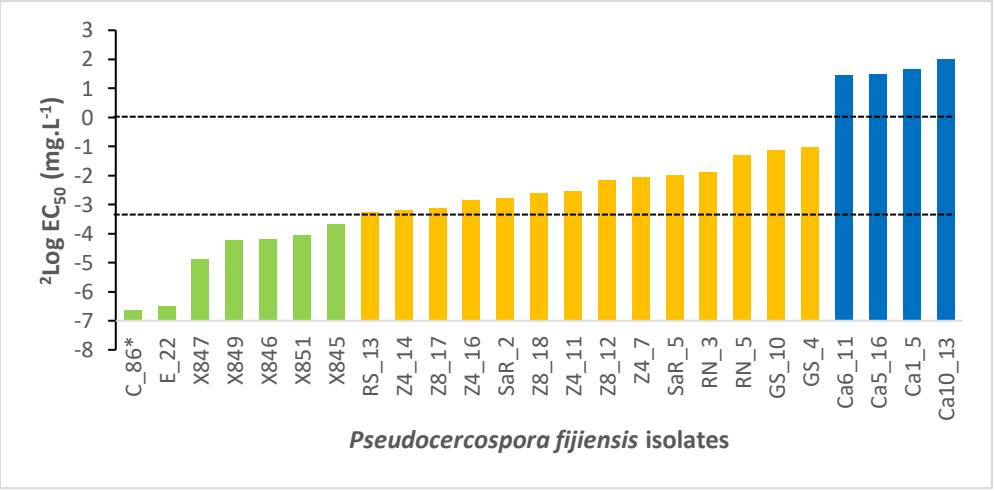


Figure 1. Sensitivity of *Pseudocercospora fijiensis* strains to propiconazole. The sensitivity thresholds are marked with dotted lines. Sensitive strains from different origin are inhibited at very low concentrations (green bars). The other strains were obtained from various banana plantations in Ecuador (E, RS, SaR, RN and GS) and Costa Rica (Ca and Z) where black Sigatoka disease is controlled through frequent fungicide applications. Tolerant *Pseudocercospora fijiensis* isolates are shown in orange. The "Ca" strains originate from the Costa Rican Cartagena banana plantation, which is frequently sprayed with fungicides, and they display the lowest level of sensitivity (blue bars).

Table 1. Origin and characteristics of the *Pfcyp51* gene and its promoter in 25 *Pseudocercospora fijiensis* strains used in this study, including their sensitivity to propiconazole (EC_{50})

Origin	Isolate	Promoter insertions	Element present	CYP51 modulations												Propiconazole EC_{50} ($mg.L^{-1}$)
Cameroon	C_86*	WT	1													0.010
Ecuador	E_22	WT	1	T18I		V106D										0.011
Taiwan	X847	WT	1			V106D			K171R			A446S				0.034
Burundi	X849	WT	1			V106D										0.053
Philippines	X846	WT	1	T18I		V106D	V116L									0.055
Gabon	X851	WT	1			V106D										0.060
Indonesia	X845	WT	1	T18I	Y58F	V106D										0.079
Ecuador	RS_13	WT	1	T18I		V106D				A313G					Y463N	0.103
Costa Rica	Z4_14	WT	1	T18I		V106D				A313G					Y463D	0.108
Costa Rica	Z8_17	WT	1	T18I		V106D				A313G					Y463S	0.114
Costa Rica	Z4_16	WT	1	T18I		V106D					A381G			G462A		0.138
Ecuador	SaR_2	WT	1	T18I		V106D				A313G			Y461D			0.147
Costa Rica	Z8_18	CTCGTACGATAGCACAAATGTTAAA TCTCGTACGATAGC	3	T18I		V106D		Y136F							Y463D	0.162
Costa Rica	Z4_11	WT	1	T18I		V106D				A313G					Y463H	0.171
Costa Rica	Z8_12	CTCGTACGATAGCACAAATGTTAAA TCTCGTACGATAGC	3	T18I		V106D		Y136F							Y463D	0.222
Costa Rica	Z4_7	WT	1	T18I		V106D				A313G					Y463S	0.241
Ecuador	SaR_5	WT	1	T18I		V106D				A313G					Y463N	0.251
Ecuador	RN_3	WT	1	T18I		V106D				A313G					Y463H	0.271
Ecuador	RN_5	WT	1	T18I		V106D				A313G					Y463H	0.404
Ecuador	GS_10	WT	1	T18I		V106D				A313G					Y463N	0.458
Ecuador	GS_4	WT	1	T18I		V106D				A313G					Y463N	0.485
Costa Rica	Ca6_11	AAATCTCGTACGATAGCATAAAATCT CGTACGATAGCATAAAATCTCGTAC GATGTTAAATCTCGTACGATAGCATA AATCTCGTACGATAGCACCTGCC	6	T18I		V106D		Y136F							Y463D	2.75
Costa Rica	Ca5_16	AAATCTCGTACGATAGCATAAAATCT CGTACGATAGCATAAAATCTCGTAC GATGTTAAATCTCGTACGATAGCATA AATCTCGTACGATAGCACCTGCC	6	T18I		V106D		Y136F							Y463D	2.776
Costa Rica	Ca1_5	CTCGTACGATAGCACAAATGTTAAA TCTCGTACGATAGC	3	T18I		V106D		Y136F			A381G				Y463D	3.16
Costa Rica	Ca10_13	AAATCTCGTACGATAGCATAAAATCT CGTACGATAGCATAAAATCTCGTAC GATGTTAAATCTCGTACGATAGCATA AATCTCGTACGATAGCACCTGCC	6	T18I		V106D		Y136F							Y463D	3.971

Resistant *Pseudocercospora fijiensis* strains always contain repetitive elements in the *Pfcyp51* promoter

Closer examination of the promoter of the *Pfcyp51* gene revealed that sensitive isolates contain a 19 bp promoter element "TAAATCTCGTACGATAGCA" (Figure 2). This element is present as a single element in the CIRAD86 reference and originally located a few nucleotides downstream in the promoter MYCFIscaffold_7:2121794 – 2121813, (-122 bp upstream of the *Pfcyp51* start codon).

A detailed analysis of the promoter of the resistant strains identify a region of high variation, with insertions starting at position at 2,121,774 of scaffold 7 in the genome sequence of the reference strain (*Pseudocercospora fijiensis* v2.0, JGI), ~103 bp upstream of the start codon of *Pfcyp51* (antisense direction). Some isolates contain a partial construction of the element in their insertions, while others have a modified element due to a few additional nucleotides. Additional to the 19 bp element of a partial construction element of 16 bp (TAAAATCTCGTACGAT) and a modify element of 20 bp (TAAAATCTCGTACGATAGCA), were also present. For example, in highly resistant strains Ca1_5, Ca5_16, Ca6_11, and Ca10_13 (Figure 2) this element is repeated up to six times (four fully conserved and one partial, mostly in tandem insertion) and three tandem times in the tolerant *P. fijiensis* strains Z8_12 and Z8_18. DNA sequence analysis of the most resistant strains from Costa Rica (Ca5_16, Ca6_11 and Ca10_13), revealed that these contain identical mutations in the coding region of the *Pfcyp51* gene, and that the length of the insertion in the promoter reach 100 bp (Table 1).

Figure 2. Sequence logo of the *Pfcyp51* promoter repeat element. Sequences of all repeat elements were aligned and used to generate a sequence logo using the WebLogo website (http://weblogo.berkeley.edu/logo.cgi). The logo displays the frequency of the nucleotides within the three different repeated elements with 16, 19 or 20 bp that we observed in the promoter. Nucleotide frequency is scaled relative to the information content (measure of conservation) at each position. The positions 3-16 are most characteristic for the repeat element**.**

Repetitive elements in the promoter of *Pfcyp51* upregulate its expression

In order to test whether *Pfcyp51* gene expression is affected by the presence of repetitive element, we performed quantitative real time RT-PCR on total RNA from mycelia, normalized to the expression of the actin gene, *Pfact. Pseudocercospora fijiensis* strains Ca5_16, Ca6_11 and Ca10_13, which have six repetitive units in their promoter, have a five-fold increase in *Pfcyp51* gene expression as compared to strains E22 and CIRAD86, that have only one (Figure 3). In contrast, no significant difference was found between the control strains and *P. fijiensis* strain Z8_12, with three units. The up-regulation of *Pfcyp51* was constitutive and independent of addition of propiconazole in the culture medium (data not shown).

High frequency of the repetitive element in reduce sensitive strains from Costa Rican banana plantations

To identify the copy number of the repetitive element present in the promoter of *Pfcyp51*, we performed PCR analysis on 225 isolates originating from four banana plantations in Costa Rica that were previously studied (Arango et al., 2016): three plantations (Cartagena, Zent and San Pablo) with intensive fungicide applications and one plantation (wild type; wt)

(ZTSC) that has not received any fungicide applications. Examination of the amplicon sizes by gel electrophoresis revealed banding patterns that correspond to two, three and six promoter repeats (Figure 4A).

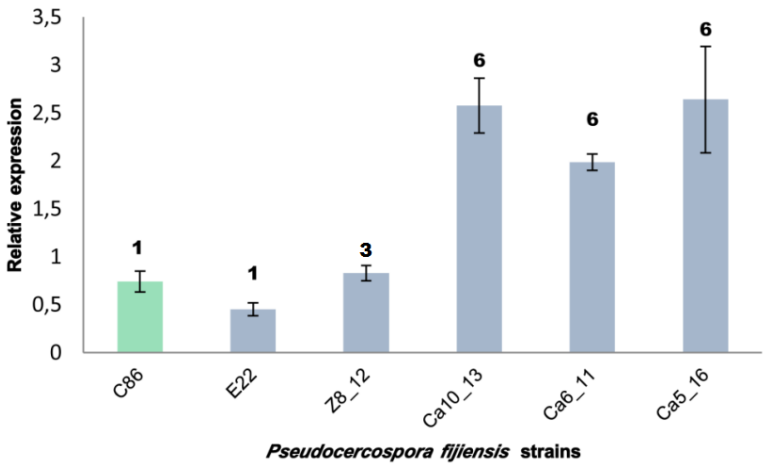


Figure 3. Relative expression of the *Pfcyp51* gene in six *Pseudocercospora fijiensis* strains carrying different numbers of the promoter element. Relative expression was normalized with the *P. fijiensis* actin gene. Numbers on top of each bar stand for the number of promoter element present. Reference isolate CIRAD86 (C86) is shown in green. Data represent averages of three biological repetitions with each at least three technical replicates (error bars indicate standard variations).

Amongst *P. fijiensis* populations collected from fungicide treated plantation, the Cartagena population was dominated by isolates containing six *Pfcyp51* copies of the element, (50 out of 82) followed by isolates with two copies (29 out of 82), isolates carrying the unique element were the least represented (3 out of 82). In contrast, Zent population was dominated by strains carrying the unique element (59 out of 84) but isolates containing two and six promoter repeats were also found (11 and 14 out of 84 respectively). San Pablo population was dominated by isolates carrying three promoter repeats (23 out of 43), this genotype was not observed in the Cartagena and Zent populations, followed by isolates with six (10 out of 23), one (8 out of 23) and two (2 out of 23) promoter repeats. In contrast, the population from

untreated plantations exclusively contained strains with just one 19 bp element in the *Pfcyp51* promoter (Figure 4B).

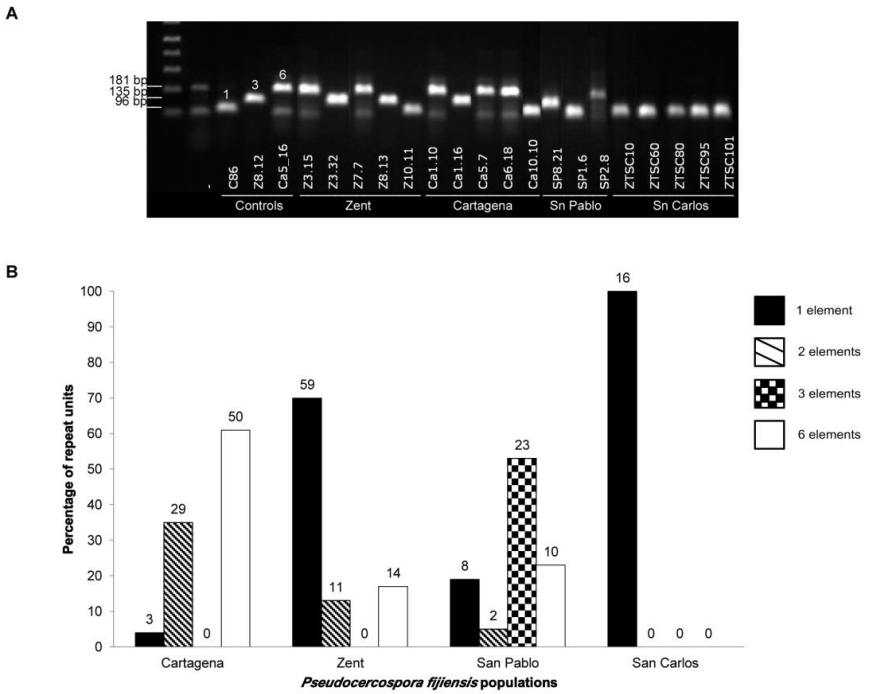


Figure 4. Screening for the *Pfcyp51* promoter repeats in *Pseudocercospora fijiensis* strains from four Costa Rican populations. A) Example of PCR amplification of *Pfcyp51* promoter in isolates from different populations**.** Isolate CIRAD86 (C86) was used as indicative control for the presence of one promoter element, Z8.12 as control with three element repeats and Ca5_16 as control of six element repeats. The number of elements repeated in each control sample is showed above the corresponding band. The other strains originate from banana plantations under fungicide disease management and represent various promoter length variants as controls. B) Distribution of the number of *Pfcyp51* promoter inserts within Costa Rican populations of *Pseudocercospora fijiensis*, based on 225 PCR amplifications.

Subsequent sequence analysis revealed that the promoter insertions were 100 (six elements), 59 (three elements) or 42 bp (two elements) in length. Most repetitive elements are inserted at 103 bp upstream of the start codon of the *Pfcyp51* gene. As mention before some isolates contain a partial construction of the element in their insertions, while others have a modified element due to a few additional nucleotides comprising three different alternatives (element of 20 bp, 19 bp, or 16 bp). Elements of 20 bp and 19 bp only differ in one nucleotide

an extra adenine, whereas the 16 bp element represents a shorter version of the 19 bp insert (Figure 5). The 19 bp element was found alone in isolates with one, two and three copies, whereas in isolates with six copies of the repetitive element, the 19 bp element was accompanied by the 20 bp and 16 bp variants present as single units. Hence, the 19 bp element is the commonest insertion across all isolates analysed (Figure 2).

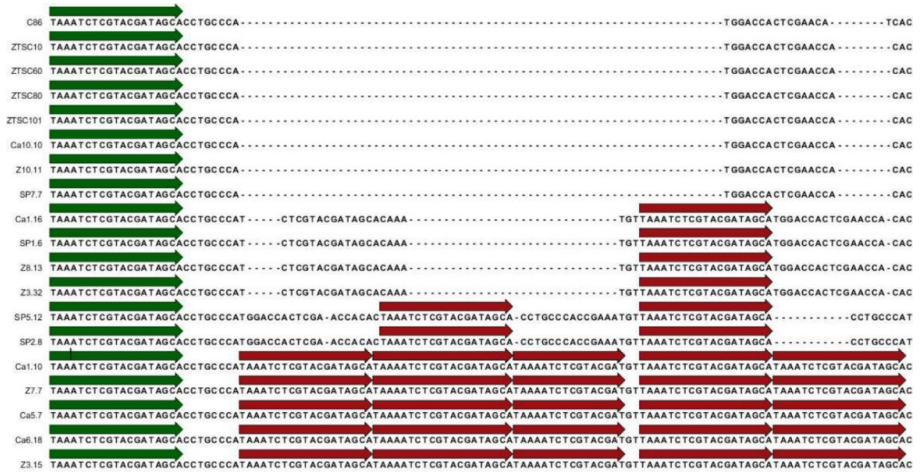


Figure 5. Alignment of the promoter region of the *Pfcyp51* gene of *Pseudocercospora fijiensis* strains from the Zent (Z), Cartagena (Ca), San Pablo (SP) and the wt San Carlos (ZTSC) banana plantations in Costa Rica. Isolate CIRAD86 (C86) is the reference wt isolate. The normal element present in all isolate at position -122 bp is shown in green arrows. The different repeated insertions found in some *P. fijiensis* isolates are shown in red.

Analysis of the *Pfcyp51* coding sequence

As expected, sequence analyses of different isolates revealed the presence of non-synonymous mutations in the coding region of *Pfcyp51*. These resulted in the amino acid (aa) changes Y136F, A313G, Y463D/H/N that were previously reported and linked to sensitivity loss for propiconazole (Cañas-Gutiérrez et al., 2009). In addition, nine not previously described aa changes (T18I, Y58F, V106D, V116L, K171R, A381G, A446S, G462A, and Y463S) were detected (Table 1). In all isolates T18I and V106D were identified. Excluding these, the most frequent aa changes were A313G and Y463N/D/S/H, present in 44% and 66% of the analysed isolates, respectively. These were often found in combination with Y136F and

A381G. The most frequent haplotype amongst the 25 isolates was T18I, V106D, Y136F, A313G, Y463D/N/S, which was found present in combination with two, three or six copies of the repetitive element and accounts for 30% of the isolates. In addition, several other combinations of aa substitutions were observed in the analysed cohort of *P. fijiensis* strains, including A313G - Y463S/H/D/N, G381A - G462A, Y136F - Y463D, Y136F - A381G - Y463D, and K171R - A446S.

Functional analysis of the *Pfcyp51* promoter insertions

We discovered a range of promoter insertions exclusively in *P. fijiensis* populations from treated banana plantations. The promoter insertions, in particular the six repeats insertion was shown to confer enhanced expression of *Pfcyp51*. The strains carrying the insertions display reduced sensitivity to DMI fungicides but, also carry *Pfcyp51* mutations in the coding sequence which is the most common mechanism for conferring shifted sensitivities to these fungicides. To disentangle the relation between both mutations in the coding sequence and the promoter insertions, we introduced the *Pfcyp51* promoter from the resistant *P. fijiensis* strain Ca5_16, (Costa Rica, Table 1) which has six repetitive elements into the sensitive wt E22 strain from Ecuador (Table 1). Transformation of *P. fijiensis* strain E22 resulted in 250 green fluorescent protein (GFP) and hygromycine (HGH) positive transformants (Figure 6A). The transformants were PCR characterized to identify strains with the six repeat elements promoter region inserted at the correct integration site from ectopic transformants (Figure 6B). Two independent transformants, Swap26 and Swap121 (Figure 6C), showing the Ca5_16 promoter amplicon (Figure 6B) and positive for the correct integration site (Figure 6C) were selected for further analyses. Subsequently, we performed qRT-PCR analyses on Swap26 and Swap121 along with the *P. fijiensis* control strains comprising the recipient wt strain E22 and the wt resistant strains Ca5_16 and Ca10_13 and an ectopic transformant.

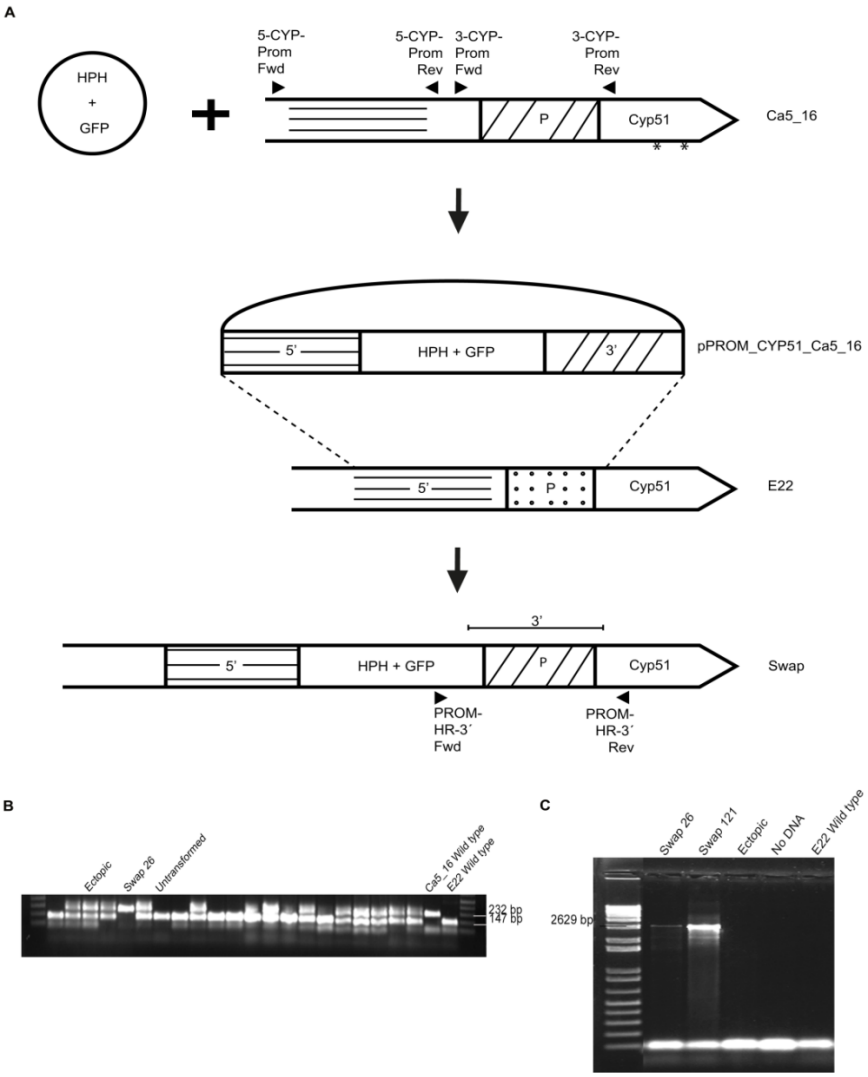


Figure 6. Transformation design for *Pfcyp51* promoter swap strains of *Pseudocercospora fijiensis.* A) Strain Ca5_16 is the *Pfcyp51* promoter donor (slashed area) in the 3´recombination fragment together with 5´fragment (crossed out area) was amplified with CYP-Prom primers and ligated to a cassette with the HGH and GFP markers into construct pPROM_CYP51_Ca5_16. This construct was inserted into the *P. fijiensis* recipient E22 sensitive strain, containing a single promoter element (dotted area). After *Agrobacterium*-mediated transformation and selection for *gfp* tagged strains, homologous recombination sites were amplified with PROM-HR-3´primers to detect and characterize promoter swapped transformants. **B**) The promoter lengths of positive GFP tagged transformants was amplified and compared with the donor and the wt recipient strain. Transformant Swap 26 is shown as an example of a true promoter replacement transformant, which show a similar amplicon as the donor strain. Ectopic transformants possess the promoter fragment of both the donor and the recipient strain, respectively, whereas untransformed strains only show the wt-sized amplicon. **C**) Verification of swapping by amplification of the 2,629 bp cassette between the homologous recombination sites and the *Pfcyp51*coding region using primers PROM-HR-3´.

Consistent with previous results, the resistant strains Ca5_16 and Ca10_13 express *Pfcyp51* at a higher level than the E22 recipient strain. Moreover, the expression of *Pfcyp51* was significantly increased in both Swap26 and Swap121 compared to E22 and the ectopic strain. The expression phenotype of both Swap26 and Swap121 was not significantly different from that of the resistant donor strain Ca5_16 (Figure 8A). Hence, these results prove that replacing the *Pfcyp51* promoter from a resistant strain to a sensitive strain results in over expression of *Pfcyp51*.

To determine whether the observed effect was independent of azole fungicides we challenged the transformants with difenoconazole, epoxiconazole and propiconazole, and calculated the EC_{50}. A consistent pattern of growth was observed on the plates. The resistant Ca10_13 strain up to concentration of 2.56 $mg \cdot L^{-1}$ of difenoconazole or epoxiconazole, and 10 $mg \cdot L^{-1}$ of propiconazole. The sensitive strain E22 and the ectopic transformant only grew up to concentration of 0.016 $mg \cdot L^{-1}$ of difenoconazole and 0.04 $mg \cdot L^{-1}$ of epoxiconazole or propiconazole. The Swap26 and Swap121 transformants grew at least on fourfold higher concentrations as compared to the sensitive wt strain E22. The ectopic transformant, displayed similar sensitivity to E22 regardless of the fungicide used (Figure 8B and 8C; Table 2). For difenoconazole, transformants Sawp26 and Sawp121 displayed a twofold and over fourfold (4,25) increment of EC_{50} compared to the sensitive E22 strain, whereas the resistant strain Ca10_13 was 703-fold more resistant. For epoxiconazole, Swap26 displayed a 4.48-fold reduction in sensitivity, while Swap121 displayed a slightly higher shift of 8.36-fold. By contrast the resistant strain Ca10_13 was 185.84-fold less sensitive to epoxiconazole than wt strain E22. The EC_{50} value for propiconazole of this strain was 4.65- and 5.23-fold higher compared to Swap26 and Swap121, respectively. The resistant strain Ca10_13 was 217.42-fold less sensitive in comparison with wt E22 (the resistant strain Ca5_16 was not analysed at

this point due to contamination). Overall this data confirms the contribution of promoter modifications in the overall sensitivity shift to the azoles in *P. fijiensis.*

Table 2. Means of EC_{50} values[1] ($mg.L^{-1}$) of the *Pseudocercospora fijiensis* promoter swapped transformants Swap26 and Swap121 and various control strains to three azole fungicides.

Sample	Difenoconazole	Epoxiconazole	Propiconazole
Ca10_13 (Resistant)	5,629 ± 0,1789	4,646 ± 0,1818	5,653 ± 0,1905
E22 (Sensitive)	0,008 ± 0,0009	0,025 ± 0,0014	0,026 ± 0,0012
Swap 26	0,016 ± 0,0062	0,112 ± 0,0205	0,121 ± 0,0228
Swap 121	0,034 ± 0,0010	0,209 ± 0,0450	0,136 ± 0,0370
Ectopic	0,003 ± 0,0001	0,023 ± 0,0011	0,014 ± 0,0016

[1]Data represent at least three independent biological replicates with each two technical repeats.

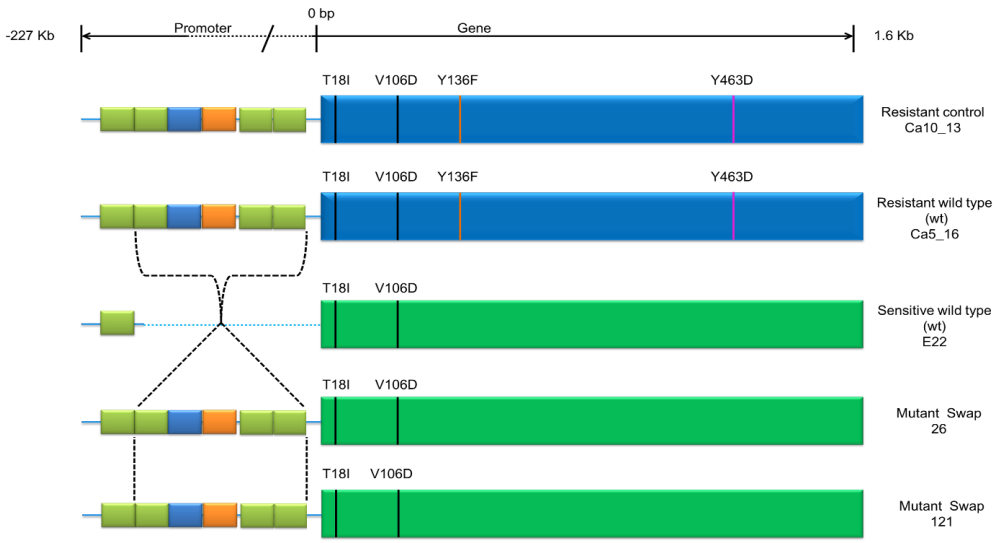


Figure 7. Configuration of the *Pfcyp51* promoter of the *Pseudocercospora fijiensis* strains used for transformation and the recombinant individuals. The promoter region is represented at the left as a blue line with different coloured boxes. Green boxes represent the 19 bp promoter repeat element. Blue and orange boxes represent alteration of 20 bp and 16 bp element respectively. Rectangular boxes at the right represent the coding region. The sensitive wt configuration is depicted in green and the resistant donor (resistant wt) configuration is shown in blue. Vertical lines –with aa substitutions - in these blue and green rectangular boxes represent mutations in the coding region.

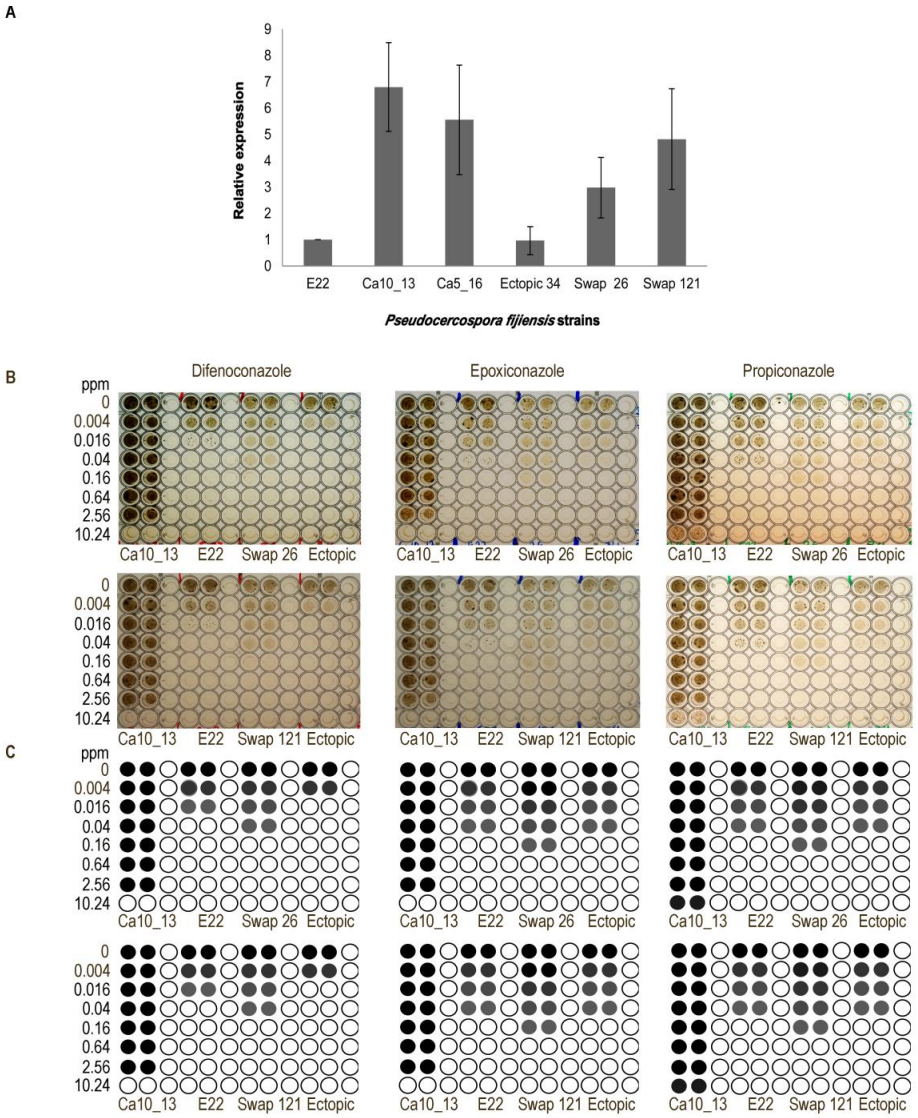


Figure 8. *In vitro* sensitivity of the promoter swapped *Pseudocercospora fijiensis* transformants Swap26 and Swap121 vs. various control strains. (A) The relative expression (normalized with the expression in wt sensitive donor strain E_22) of *Pfcyp51* in Swap26 and Swap121, the wt E22 and the resistant strain (Ca10_13) with identical promoter and coding region as donor strain (Ca5_16) as well as the ectopic control strain (Ectopic 34). Data represent the averages of three replications. (B) Fungicide sensitivity assays of Swap26 and Swap121 and the ectopic, wt resistant (Ca10_13) and recipient (E_22) controls to 0 – 10.24 ppm of difenoconazole, epoxiconazole and propiconazole at 10 days post inoculation (pictures are representative for three independent repetitions). (C) Graphical interpretation of the fungicide sensitivity assays shown in (B).

Discussion

Management of crop diseases is commonly based on an integrated approach making use of combined breeding for host resistance, agronomic measurements and crop protection agents whenever necessary (Matthews *et al.* 2014). Due to the overall *P. fijiensis* susceptibility and ubiquity of "Cavendish" clones, which represent over 90% of the global banana trade, disease control in banana is almost entirely relying on crop protection agents and prophylaxis measures. Despite the use, under particular conditions, of forecast and disease monitoring as decision support systems, accompanied with prophylaxis measures as leaf surgery and removal of infected material to reduce the inoculum potential, the cornerstone for *P. fijiensis* control remains chemical crop protection (Chong et al., 2016a). Consequently, the selection pressure on the pathogen has been enormous, which resulted in the appearance of fungicide resistant populations. This urges for a better understanding of the nature and development of resistance.

Known mechanisms of resistance against azole fungicides include non-synonymous point mutations in the *cyp51* coding region, overexpression of the gene and the overexpression of membrane efflux pumps (Ma et al., 2006; Stergiopoulos et al., 2002). A number of mutations in the c*yp51* gene that are linked to DMI resistance are shared across diverse species and some are linked to a specific azole (reviewed by Becher and Wirsel, 2012). In the case of *P. fijiensis*, the presence of mutations in the *Pfcyp51* gene was related to propiconazole resistance (Cañas-Gutiérrez et al., 2009). In the present work we have focused on the promoter region as an important determinant for *Pfcyp51* gene expression, and describe the identification of a 19 bp repetitive element, whose presence upregulates *Pfcyp51* expression and leads to reduced DMI sensitivity. Our data represent the first report of targeted genetic manipulation in *P. fijiensis*, and the first description of a modified promoter resulting in the

over expression of *Pfcyp51* and contributing to reduced DMI sensitivity, thereby constituting a new mechanism of DMI resistance in this organism.

We observed a broad sensitivity range among the different *P. fijiensis* strains to the tested DMI fungicides with a clear connection between geographical origin of the strains and reduced sensitivity to these compounds. This is in agreement with previous work showing that the majority of resistant strains was isolated from countries where the banana production is economically very important, such as Costa Rica and Ecuador, and where fungicide application frequencies are very high (Aguilar-Barragan *et al.* 2014; Amil *et al.* 2007; Arango *et al.* 2016; Chong *et al.* 2016b; Marín *et al.* 2003).

The majority of *P. fijiensis* isolates from the Zent population were tolerant, whereas the strains from the Cartagena population were amongst the most resistant encountered in this study. Interestingly, sensitive strains were still found in these heavily treated plantations and, more surprisingly, some strains from the non-treated ZTSC population showed tolerance or resistance (Chong et al., 2016a). Despite this pattern was observed in very small portion of these populations, it raises questions about the underlying mechanisms. We tentatively propose that this could be due to a low frequency gene flow according to the stratified dispersal combination with the relatively narrow spatial scale of ascospore distribution (Rieux et al., 2014, 2013) for the approximately 100km distance between ZTSC and the other locations (Arango et al., 2016).

Pseudocercospora fijiensis populations in banana plantations in Costa Rica that are frequently sprayed with fungicides comprise a plethora of genotypes with diverse mutations in the coding region of the *Pfcyp51* gene (Chong et al., 2016b). Some of these mutations were previously reported in Colombian *P. fijiensis* populations and were related with resistance to propiconazole (Cañas-Gutiérrez et al., 2009) as well as to other azoles in *Zymoseptoria tritici*, *Candida albicans*, and *Aspergillus fumigatus* (Akins and Sobel, 2009; Cools et al., 2013;

Mellado et al., 2007). These aa changes are all located at the SRS (Alvarez-Rueda *et al.* 2011; Becher & Wirsel 2012; Morio *et al.* 2010; Mullins *et al.* 2011).

One of the most frequent aa substitutions found in our work, Y136F, was previously reported for *Blumeria graminis* (Wyand & Brown 2005), and for *C. albicans* (Morio *et al.* 2010). Changes in the Ca5_16 and Ca10_13 strains are equivalent to Y137F, A379G and Y461 in *Z. tritici*, which are related to different and highly resistant azole phenotypes (Leroux & Walker 2011; Stammler *et al.* 2009). The substitution Y137F is close to the azole docking site, A379 forms part of the secondary structure adjacent to the cavity, and Y461 is located at the heme end(Mullins et al., 2011). For many of the isolates we eventually had only DNA available, as *P. fijiensis* isolates are hard to maintain, hence there was no possibility to examine for the DMI sensitivity phenotypes in all the haplotypes. However, this will be addressed in a wider study in the future (Chong et al., 2016b).

Unexpectedly, we found that in addition to the *Pfcyp51* coding region mutations, the majority of the *P. fijiensis* strains from the Costa Rican Cartagena population contain a 100 bp insertion in the promoter region. These insertions are composed of six copies of a repetitive element, whereas a single copy of this element is present in all sensitive strains. Strains with reduced sensitivity have usually two, three or more copies of this element. Changes in the promoter region of the *cyp51* gene have been described in other fungi, such as truncated derivatives of a LINE-like retrotransposon in *Blumeriella jaappi* (Ma et al., 2006), a MITE-like transposon named PdMLE1 in *Penicillium digitatum* (Sun *et al.* 2013), a larger transposon of 1.8 kb in *A. fumigatus* (Albarrag et al., 2011; Verweij et al., 2013) and transcription factors binding site in *V. inaequalis* (Villani et al., 2016). More detailed studies would be required in *P. fijiensis* to decipher whether the insertions we observed corresponds to the movements of a transposon sequence or whether the *Pfcyp51* expression might also be regulated by transposons. However, unlike previous reports of promoter insertions with a 199 bp to 5.6 kb-

sequence transposon, the promoter insertion in *Pfcyp51* is a repeated merely 19 bp fragment, reaching only 100 bp in length, even shorter than insertions in *Venturia inaequalis* (Schnabel and Jones, 2001; Villani et al., 2016) and *Z. tritici* (Cools et al., 2012), where transposons were not reported. In other organisms e.g. *E. coli*, overexpression of a desired gene was achieved by tandem repeats of core promoter sequences called "MCP*tac*s" (Li et al., 2012).

Repeated elements in the *ERG11* promoter sequence from *Z. tritici*, were suggested to have appeared after the initial mutations in the coding region. In this way, a larger accumulation of mutations could be avoided, that would compromise the activity of the enzyme (Cools *et al*. 2012; Leroux & Walker 2011), but however, contribute further to sensitivity reduction. Possibly, this also applies to *P. fijiensis*, for which we did not find tolerant or resistant isolates with insertions in the promoter and no mutations within the coding region. Isolates from wild populations lacked promoter insertions, but - occasionally - possessed mutations within the coding region. Thus far, we do not have any indication for promoter insertions being driven by sexual recombination (Chong et al., 2016c).

We studied the regulatory nature of the inserted sequences in *P. fijiensis in silico* and show that the 19 bp (TAAATCTCGTACGATAGCA) repetitive element is the most common feature. Using a targeted reverse genetics approach in *P. fijiensis* we for the first time could validate that the presence of six copies of this element in the *Pfcyp51* promoter increases the expression of *Pfcyp51* at least five-fold, compared to wt strains and those with one or three elements of tolerant phenotypes. Previously, Cañas-Gutiérrez et al. (2009) were unable to show such expression in experiments with *P. fijiensis* in response to propiconazole and considered it either a non-existent or unimportant mechanism in this fungus. However, this can be explained by a smaller data set and the fact that those strains had a much higher sensitivity than the strains in our study. Hence, we now propose that promoter repeats constitute a genetic adaptation mechanism to the high selective pressure imposed on *P. fijiensis*

by the repeated use of different DMI fungicides, particularly since this same phenomenon has been observed in various geographically discontinuous populations, including the Philippines, Cameroon, Colombia and Costa Rica (Chong et al., 2016b).

Within population, we identified a clear genetic diversity in the number of promoter repeats. The frequency of strains with more repeats was higher in banana plantations with up to 8 DMI cycles sprayed, such as Cartagena, Zent and San Pablo. Strikingly, all isolates from the untreated San Carlos plantation contained the single 19 bp element present in sensitive wild isolate around the world. These data provide additional evidence that the promoter insertions constitute an adaptation mechanism to fungicide applications in banana plantations.

Even though *P. fijiensis* is a difficult fungus to transform (Díaz-Trujillo *et al.* 2016b), and despite site specific recombinations levels seem to be very low, promoter swapping was successfully applied in our study. The introduction of the promoter from a resistant *P. fijiensis* strain into a sensitive isolate by site specific recombination resulted in a transformant with increased expression of *Pfcyp51*, and consequently reduced sensitivity to three azole fungicides, as a result of the promoter replacement. The Swap26 and Swap121 transformants were at least four times less sensitive than the recipient wt strain E22, but not as resistant as the resistant strains Ca10_13 or the donor strain Ca5_16 which were both carrying similar (Y136F and Y463D) mutations in the coding region. From our results it is expected that the reverse experiment, swapping the promoter from the resistant strain for a wild type promoter should lead to reduced resistance. Finally swapping the wt *Pfcyp51* gene into a resistant strain but keeping the insertions might reveal differential genetic backgrounds between sensitive and resistant strains that might contributed to the sensitivity. The combination of both mechanisms: 1) overexpression conferred by promoter insertions and 2) target mutation likely explain most of the shift towards DMIs.

DMIs are and will likely remain a cornerstone for global black Sigatoka disease management. However, the risks of bad practices and too frequent applications are considerable since they exert a significant selection pressure on *P. fijiensis* populations, turning these increasingly more resistant. Hence, DMI applications are, may lose their competitive advantage compared to other less environmentally friendly compounds. The practical spin-off of this study is that we now can use a simple PCR assay to monitor, evaluate and predict reduced DMI sensitivity in *P. fijiensis* field populations.

Evidently, DMIs are under pressure due to resistance and therefore increasingly being studied in various fungal pathogens, including *P. fijiensis.* This fosters efforts into the research and development for novel chemistry for efficient black Sigatoka control, although alternative products, such as the strobilurins and Succinate Dehydrogenase Inhibitors (SDHIs), are also prone to resistance development (Arango *et al.* 2016; Scalliet *et al.* 2012). Therefore, disease management should embark on the availability of resistant banana germplasms. Nonetheless, disregarding of which banana cultivars dominate the export trade, fungicide resistance monitoring and the strict adoption of use recommendations of the products is an absolute necessity.

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Chapter 5

Genetic mapping of resistance to 14α-demethylase inhibitor fungicides in the banana black Sigatoka pathogen *Pseudocercospora fijiensis* reveals *Pfcyp51* as a single explanatory factor

Pablo Chong, Aikaterini Vichou, Henk Schouten, Harold J. G. Meijer, Rafael Arango and Gert H. J. Kema

Abstract

The haploid fungus *Pseudocercospora fijiensis* causes black Sigatoka in banana and is chiefly controlled by extensive fungicide applications, threatening occupational health and the environment. The 14α-Demethylase Inhibitors (DMIs) are important disease control agents, but they lose sensitivity in a rather gradual fashion, suggesting an underlying polygenic genetic mechanism. Evidence found thus far suggests that *P. fijiensis cyp51* gene is the single responsible factor for sensitivity loss in the field. In this study we performed molecular analysis, including the construction of genetic maps, to better understand the mechanisms involved in DMI resistance in *P. fijiensis*. Two different DMI resistant *P. fijiensis* strains were crossed with a sensitive strain. Analysis of the inheritance of DMI resistance in the two F_1 populations revealed a strong bimodal distribution, indicative of a single major responsible gene. Based on the bimodal distribution, the causal factor was genetically mapped as a single factor, using DArTseq markers and DMI-sensitivity scorings of both F1 populations. This results in the generation a genetic linkage maps for each population. Both maps indicated a similar genetic region on the resistant parents harbouring the responsible factor for DMI resistance. Full agreement was found for genetic markers in either population, underlining the robustness of the approach. The two maps indicated a similar genetic region where the *Pfcyp51* gene is found. Sequence analyses of the *Pfcyp51* gene of the F_1 populations also revealed a matching bimodal distribution with the DMI resistant. Amino acid substitutions in *P. fijiensis* CYP51 enzyme of the resistant progeny were previously correlated with the loss of DMI sensitivity. In addition, the resistant progeny inherited a *Pfcyp51* gene promoter insertion, composed of a repeat element with a palindromic core, also previously correlated with increased gene expression. This genetic approach confirms that *Pfcyp51* is the single explanatory gene for reduced sensitivity to DMI fungicides in the analysed *P. fijiensis* isolates.

Introduction

The dothideomycete fungus *Pseudocercospora fijiensis* (previously *Mycosphaerella fijiensis*) is the causal agent of black Sigatoka, a major global threat to banana crops that is responsible for serious economic losses in banana production and provokes major negative environmental impacts due to the current control strategies (Chong *et al.* 2016a). Contemporary disease control is mainly achieved by the application of systemic fungicides of which the most commonly used fungicides belong to the 14α-Demethylase Inhibitors (DMIs) group. DMI are single target fungicides, hence, sensitive to resistance development. Fungicide application frequencies for black Sigatoka management are extensive and DMIs are important constituents of the spray schedules, which have not only serious negative environmental and social impacts, but also contribute to the development of resistance in the pathogen populations (Beaglehole *et al.* 2003; Guzmán *et al.* 2013; Marín *et al.* 2003). In general, most microorganisms adapt to fungicides by the selection of individuals with modulated genetic information. Commonly observed genetic mechanisms resulting in reduced DMI sensitivity in *P. fijiensis* are point mutations in and overexpression of 14α-demethylase that is encoded by the *Pfcyp51* gene (Bolton *et al.* 2016; Cañas *et al.* 2009; Chong *et al.* 2010; Chong *et al.* 2016b; Churchill 2011a; Díaz-Trujillo *et al.* 2016a; Lepesheva & Waterman 2004).

Abrupt loss of fungicide efficacy in the field is usually considered to be monogenic, resulting from mutations in a single major gene. As a result, the pathogen subpopulation carrying the mutation(s) becomes dominant and higher fungicide concentrations do not enable improved disease management, also indicated as qualitative resistance. The resistance to strobilurins in various plant pathogenic fungi, including *P. fijiensis,* illustrates this observation (Arango *et al.* 2016). In contrast, quantitative and hence, gradually shifting reduced sensitivities are enabled by the interaction of a number of different genes (Dyer *et al.* 2000). DMI resistance mechanisms in fungi have a quantitative polygenic nature. In *Candida*

albicans, Aspergillus fumigatus and *Zymoseptoria tritici* DMI resistance involves modification of sterol biosynthesis and increased expression of membrane transporters, e.g. ATP-binding cassette transporters and major facilitators, resulting in modified fungicide efflux that leads to reduced efficacy (Cools *et al.* 2013; Cowen 2008). All current evidence in *P. fijiensis* points to *Pfcyp51* as a major factor responsible for reduced sensitivity to DMIs. However, the loss of sensitivity to DMIs in the field has been gradual in nature (Cañas *et al.* 2009; Marín *et al.* 2003) and a recent study revealed extraordinary high EC_{50} values in some strains, questioning whether changes in the *Pfcyp51* gene are the only underlying genetic mechanism (Chong *et al.* 2016b). Hence, additional quantitative genetic components may exist that directly or indirectly modulate resistance.

P. fijiensis has a bipolar heterothallic mating system (Conde-Ferraez *et al.* 2007), which facilitates genetic studies by crossing strains with opposite mating types (Arango *et al.* 2016; Kema 2009). Recently, a genetic linkage map for *P. fijiensis* was generated to support genome assembly, but specific mapping studies on fungal characteristics have not been accomplished (Arango et al., 2016). The aim of the present study was to unravel the genetic basis for reduced sensitivity towards DMI's fungicides in *P. fijiensis* by objective genetic mapping using Diversity Array Technology (DArTs) markers that also were used to generate a new genome assembly. Contrary to our expectations, progeny analyses provided strong evidence that DMI resistance in *P. fijiensis* is solely based on *Pfcyp51* modulation and not on other previously reported mechanisms such as increased efflux (Becher & Wirsel 2012; Cools *et al.* 2013; Leroux & Walker 2011). Despite increasing and accumulating data on fungicide resistance in fugal human and plant pathogens (Chowdhary *et al.* 2013; Cools & Fraaije 2013; Cools *et al.* 2013; Eddouzi *et al.* 2013; Guzmán *et al.* 2013; Hollomon 2015; Sun *et al.* 2013; Verweij *et al.* 2013; Villani *et al.* 2016), our study is the first genetic analysis to map the underlying genetic factors for reduced DMI efficacy.

Materials and methods

Fungal isolation

Banana leaves with black Sigatoka symptoms were collected from untreated field plots on the island of Bohol, Philippines, and from the commercial Cartagena plantation in Costa Rica that is weekly sprayed with fungicides (Chong *et al.* 2016b). Infected leaf pieces (~2x3 cm) with mature necrotic lesions were retrieved and stapled to a circular 90 mm diameter filter paper (Whatman 113, Little Chalfont, UK). Filter papers containing four or five leaf pieces were incubated for 48 hours in humid chambers (sealed plastic container with humid cotton) and subsequently soaked in water for five minutes. The excess of water was blotted with paper towel and the filter papers were placed on the lid of inverted petri dishes filled with 1% water agar. The drop in relative humidity facilitates the discharge of ascospores and single spore isolates were recovered with a needle after one-night incubation at 4°C in a refrigerator and transferred to Petri dishes with potato dextrose agar (PDA) medium that were incubated at 27°C in the dark for three weeks.

Inoculum preparation

To prepare inoculum, a piece of mycelium (~0.5 cm^2) from a mono-ascosporic *P. fijiensis* colony (three to four weeks old) grown on PDA was blended for 20 seconds at 6,000 rpm in an Ultra Turrax Tube Drive machine using a sterile DT-20 tube (Tube with rotor stator element, IKA, Staufen, Germany) in 15 ml of distilled water (Peláez *et al.* 2006). The mycelial fragments were filtered through a Steriflip Vacuum-driven Filtration System (100 µm, Millipore, Billerica, USA) and counted with a Kova glasstic slide 10 with a grids coverslip microscope slide (Kova, California, USA) and the suspensions were diluted to a final concentration of approximately $5x10^5$ mycelial fragments.ml^{-1}.

Microtiter experiments and analyses

From the abovementioned mycelium solution, a 50 µl aliquot was transferred to each well of a 96-well microtiter plate (Corning 96-well Flat Bottom Transparent Polystyrene uncoated, Corning, USA) that were filled with 200 µl potato dextrose broth (PDB) medium with antifungal compounds. Seven compound concentrations were tested with two technical repetitions per strain. All experiments were repeated three times. The samples were incubated in the dark at 27°C for 10 days to allow the mycelium to grow. Subsequently, the microtiter plates were analysed in the Infinite® 200 PRO machine branch (TEKAN, Switzerland) at room temperature (~20°C), without cover, at a wavelength of 690 nm with multiple reads per well in a 5x5 circle-filled form to determine mycelium proliferation by optical density. The bandwidth was 9 µm with five flashes per read that started 1 mm from the well wall to prevent border effects.

Fungicide compounds

The fungicides propiconazole, difenoconazole and epoxiconazole were provided by Syngenta Crop Protection AG (Basel, Switzerland), were of technical grade quality and maintained as stock solution in DMSO (propiconazole and difenoconazole at 50,000x and epoxiconazole at 20,000x). The final testing concentrations for all compounds were 0; 0,004; 0,016; 0,04; 0,16; 0,64; 2,56 and 10,24 $mg.L^{-1}$ with 1% DMSO. The sensitivity ranges for the compounds were established by calculating the 50% effective concentration (EC_{50}) by plotting the growth profiles based on OD readings. Monotone regression spline functions (Ramsay 1988) were applied to fit the curve profiles using GenStat 18th Edition software (VSN International, Hemel Hempstead, UK). The EC_{50} thresholds for categorizing *P. fijiensis* isolates as either DMI resistant or sensitive were arbitrary chosen based on the cluster analysis of the Least Standard error of the Differences (LSD) of the $^{2}\log EC_{50}$ individual means in each

population. The selected EC_{50} thresholds were: resistant isolates >1 $mg.L^{-1}$ and sensitive isolates ≤ 0.2 $mg.L^{-1}$

Crosses between sensitive and resistant P. fijiensis strains

Five DMI resistant mono-ascosporic *P. fijiensis* field isolates from Costa Rica (CaM10_16, CaM10_6, CaM7_19, CaM7_10 and CaM10_21), maintained in the Plant Research International collection were selected for crosses with the mono-ascosporic wild type strain Bo_1 (Bohol, Philippines, Table 1). The isolates were crossed shortly after the first sensitivity assay to avoid loss of sexual fitness as experienced in numerous other tries for developing *P. fijiensis* mapping populations (no more than two sub-cultivation steps). The mating type locus (*mat)* configuration was determined using the *mat1-1* primers *Mat1F* (5'-CATGAGCACGCTGCAGCAAG-3') and *Mat1R* (5'-GTAGCAGTGGTTGACCAGGTCAT-3') and the *mat1-2* primers *Mat2F* (5'-GGCGCTCCGGCAAATCTTC-3') and *Mat2R* (5'-CTTCTCGGATGGCTTGCGTG-3') (Arzanlou *et al.* 2010). The PCR reaction was performed using Roche Taq DNA polymerase with a standard mix containing 10 ng of gDNA according to the following protocol: 94°C for 4 min., then 30 cycles 30 sec. at 94°C, 40 sec. at 62°C and 40 sec. at 72°C, followed by a final extension step for 7 min. at 72°C. As *Mat* determinations by PCR were not conclusive, we eventually decided to use the Bo_1 isolate as common parent in five pairings, which we expected to be no less than 40% successful due to the bipolar heterothallic mating system of *P. fijiensis* (Conde-Ferraez *et al.* 2007). We, therefore prepared 15 ml of mycelium solution - as described above - of each parental strain and then mixed the sensitive *P. fijiensis* strain Bo_1 in a 1:1 ratio with each of the other aforementioned Costa Rican strains, hence in total five mixtures, which were incubated overnight at 27°C to recover from blending. The next day the inoculum mixtures were atomized on individual "Cavendish" banana plants, variety Grand

Nain, using a spray device (#0267-6, Preval®, Chicago, USA) at both sides of the leaves until run-off. Each plant was six months old to ensure large leaves with more surface area for disease development. After inoculation, plants were maintained in the greenhouse for 14 weeks with the following growth regime: light (>300 µmol m^{-2} s^{-1}) period of ~12 hours; day/night temperatures of 28°C/25°C with a relative humidity >90%. First necrosis and mature spots appeared around 65 days after inoculation (dai) and starting from that day, leaf pieces with the mature reproductive lesions were taken for ascospore discharge as described above. The first set of spores was observed and collected 73 days after inoculation from two crosses (Table 1) and 100 ascospores were isolated from each cross for further analyses.

Table 1. Crossing *Pseudocercospora fijiensis.* The DMI sensitive strain (Bo_1), mating type *mat1-1,* was crossed to five *mat1-2* DMIs resistant strains (CaM10_16, CaM10_6, CaM7_19, CaM10_21) and one *mat1-1* resistant strain (CaM7_10). Crosses were performed directly after the preliminary sensitive assay to avoid possible loss of sexual fitness due to sub-cultivation.

Cross	DMI sensitive parent 1	Propiconazole EC_{50} average score ($mg.L^{-1}$)	DMI resistant parent 2	Propiconazole EC_{50} average score ($mg.L^{-1}$)	Progeny
N1	Bo_1	0.020	CaM10_16	5.730	No progeny
N2			CaM10_6	11.750	Successful cross
N3			CaM7_19	5.125	No progeny
N4			CaM7_10	2.205	Incompatible cross*
N5			CaM10_21	6.349	Successful cross

* The *mat* gene configuration was unknown in the moment of the cross experiment.

DArTseq marker generation

A set of 98 isolates from each population was genotyped using DArTseq technology (www.diversityarrays.com). DNA samples were processed as described previously with few modifications (Kilian *et al.* 2012). The technology was optimized for *P. fijiensis* by using two restriction enzymes, i.e. *PstI* and *MseI*, rather than *PstI* only. The Restriction Enzyme (RE) overhangs differed from one another, allowing ligation of RE-site specific adaptors. The *Pst*I-

compatible adapter was designed to include Illumina flow cell attachment sequence, the sequencing primer sequence and a "staggered", varying length barcode region, similar to the sequence reported by Elshire (Elshire *et al.* 2011). The reverse adapter contained the flow cell attachment region and a *Mse*I-compatible overhang sequence. Only "mixed fragments" (*Pst*I-*Mse*I) were effectively amplified by PCR. Equimolar amounts of amplification products from each sample of the 96-well microtiter plate were bulked and applied to c-Bot (Illumina) bridge PCR followed by sequencing on an Illumina Hiseq2000. Each generated marker had a sequence length of 68 bp. Sequences generated from each lane were processed using proprietary DArT analytical pipelines (Kilian *et al.* 2012). In the primary pipeline the fastq files were processed to filter away poor quality sequences, applying more stringent selection criteria to the barcode region compared to the rest of the sequence. In that way the assignments of the sequences to specific samples carried in the "barcode split" steps were very reliable. Approximately 2,000,000 sequences per barcode/sample were identified and used in marker calling. Identical sequences were collapsed into "fastqcoll files" and a second pipeline was followed for further quality selection criteria as described (Kilian *et al.* 2012). Finally, the scored markers (presence/absence of restriction fragments) were represented in a 0/1 binary matrix to be used in the calculation of the genetic similarity.

Genetic linkage maps

Approximately 5,400 DArTseq markers were generated for the segregating F_1 populations N2 and N5 (Table 1). As the DMI sensitivity trait showed a clear bimodal distribution (sensitive versus resistant), this trait was integrated as phenotypic marker in both genetic maps. The markers were filtered based on their co-segregation with the sensitivity trait and those with close linkage (<10cM) to the trait were selected for the construction of a linkage group to identify the genetic region of the responsible gene. The linkage group markers were

sorted with the genetic mapping software JoinMap 4.1 (Stam 1993) and, according to the positions of the recombination events, the strains were sorted to identify the chromosomal region harboring the sensitivity gene. Subsequently, the genetic map was linked to the physical map by aligning the DNA sequences of the DArTseq markers to the *P. fijiensis* CIRAD 86 reference genome version 2.0 (http://fungi.ensembl.org/Pseudocercospora_fijiensis_cirad86/Info/Index).

Pfcyp51 sequencing

The *Pfcyp51* genes of a total of 193 isolates from both populations (98 strains from N2 and 95 strains from N5) were sequenced. To amplify the *Pfcyp51* gene, specific primers were used: *CYP51_Pfijien_F1* (5'-AAGGTCATATCGCAGG-3') and *CYP51_Pfijien_R1* (5'-GAATGTTATCGTGTGACA-3'). A standard PCR mix was used in the following program; initiation with a 5 min. denaturation at 94°C followed by 35 cycles of 30 sec. denaturation at 94°C, 30 sec. annealing at 55°C and 90 sec. extension at 68°C with a final round of seven min. extension at 72°C. The DNA sequencing of the *Pfcyp51* amplicons was directly performed on the PCR products (Macrogen Europe, Amsterdam, The Netherlands). Full sequence coverage was obtained by using four primers: *CYP51_Mfijien_F2* (5'-ACAGAAACATCACCTCC-3', *CYP51_Mfijien_F3* (5'-ATTGCTTCACTTTCATCC-3'), *CYP51_Mfijien_F4* (5'-CTCTACCACGATCTCGAC-3') and *CYP51_Mfijien_R2* (5'-GATATGGATATAGTTGTC-3'). The obtained *Pfcyp51* sequences were assembled per strain in contigs (SeqMan application software Lasergene v8, DNASTAR®, Madison, USA) and aligned to gene model MYCFIDRAFT_30715 of the reference genome (*P. fijiensis* CIRAD 86, genome version 2.0, (Arango *et al.* 2016)), using the CLC Genomic Workbench software (version 7.5.2, CLC Bio-Qiagen, Aarhus, Denmark).

Results

Progeny generation and DMI segregation

Successful crosses were accomplished after two experimental failures where we empirically determined the critical number of sub-cultivations of the parental strains, which should not be more than two in order to maintain sexual fitness. Among the five evaluated crosses, four combinations produced mature lesions at 65 dai, but ascospores were only discharged from N2 and N5 at 73 dai (Table 1). Other crosses failed, apparently due to identical *mat* genotypes. A total of 200 progeny isolates was characterized for DMI sensitivity using epoxiconazole, propiconazole and difenoconazole (Table 2).

The segregation ratios for sensitivity versus resistance of the N2 and N5 progenies were 47:53 and 44:56, respectively, according to an expected 1:1 ratio for a single gene inheritance. Hence, examination of the sensitivity response in both F_1 populations revealed a clear bimodal distribution (Figure 1, Figures S1 and S2). Despite these bimodal distributions, four strains (N2_21, N2_89, N5_1, and N5_57) had an intermediate response to the tested DMIs (Figure 1). These four strains were included in the mapping generation. Based on the sequence analyses, only N5_1 was regarded as resistant, whereas the others were considered sensitive. The average EC_{50} scores for each strain are shown in the Table S2.

Genetic linkage maps

We used the DArTseq markers to construct two linkage maps. From population N2, 53 markers were selected with 17 markers in coupling phase to resistance and 36 markers in coupling phase to sensitivity to DMIs (Figure S4a and Table S1). The markers were clustered in seven groups based on their segregation patterns. Thirty-three of the markers were placed onto scaffold 7 of the physical map of *P. fijiensis* (Table S1). The recombination events for the seven groups in the N2 population are shown in the right panel of Figure 2. From 53

markers, 21 fully co-segregated with sensitivity to the DMIs. Markers 12410413 and 12405280 were identified as the flanking markers of the sensitivity trait with physical positions scaffold_7:1,779,092 bp and scaffold_7:2,130,447 bp, respectively, and a physical distance of 351,355 bp.

Table 2. Summary of the EC_{50} data for DMIs for the two *Pseudocercospora fijiensis* mapping populations N2 and N5. Indicated are the highest (Max) and lowest (Min) values that were obtained in the discretely segregating sensitive or resistant groups as well as their average values, the percentage of strains in each category and the average resistance factor (RF) of the resistant segregants.

		Difenoconazole					Epoxiconazole					Propiconazole				
Pop	Trait[1]	Max	Min	Av (SD)	%	RF	Max	Min	Av (SD)	%	RF	Max	Min	Av (SD)	%	RF
N2	S	0.213[2]	0.009	0.05 (0.04)	47	-	0.19	0.015	0.04 (0.04)	47	-	0.21[2]	0.027	0.07 (0.03)	47	-
	R	>10.24	5.669	8.82 (1.268)	53	187.57	>10.24	1.351	4.78 (2.08)	53	108.65	>10.24	3.732	7.91 (2.1)	53	106.90
N5	S	0.108	0.005	0.04 (0.03)	44	-	0.315[2]	0.011	0.05 (0.05)	44	-	0.273[2]	0.015	0.07 (0.04)	44	-
	R	7.664	0.597[2]	4.30 (2.21)	56	107.43	9.459	0.748[2]	5.30 (2.22)	56	98.47	7.233	0.683[2]		56	94.22

[1]Sensitivity trait: S= sensitive, R= resistant.
[2]Four strains showed intermediate phenotypes.

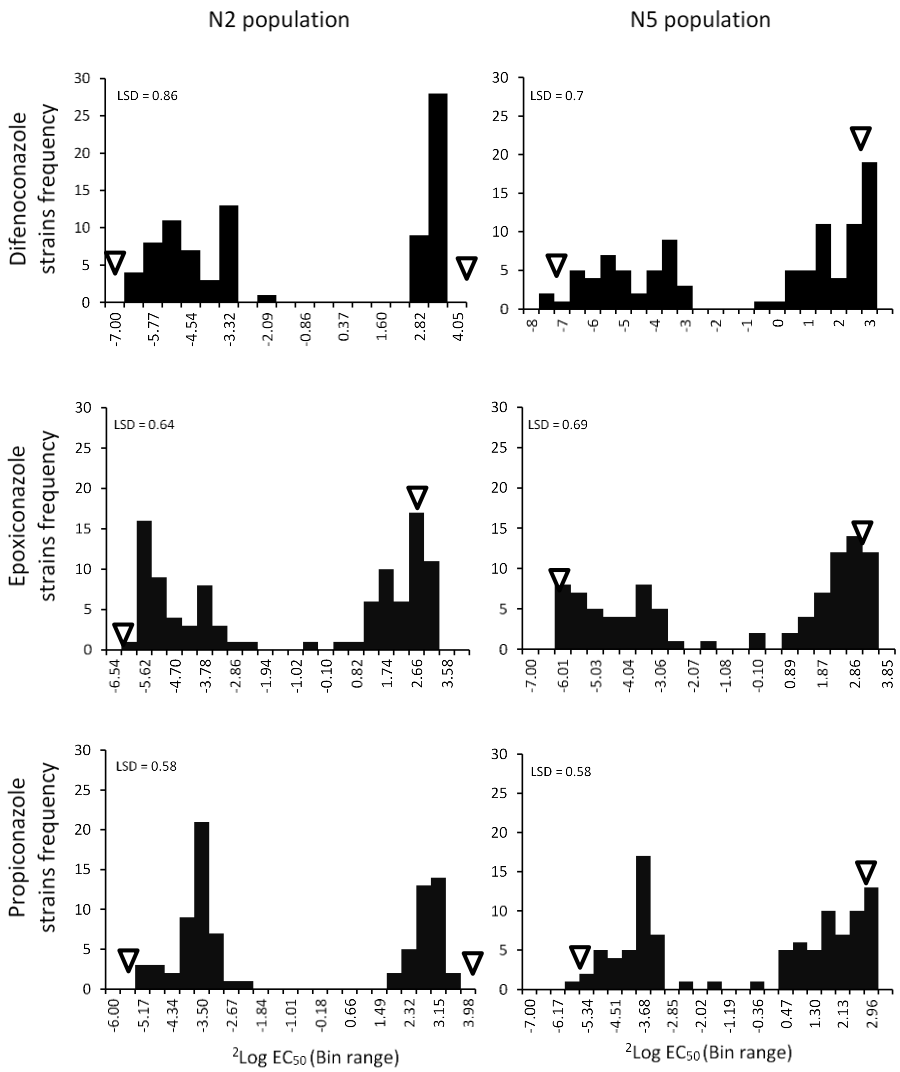

Figure 1. Segregation of DMI sensitivity in the *Pseudocercospora fijiensis* mapping population N2 and N5. Histograms of the ^{2}log average EC_{50} data per fungicide are shown for each population. Resistant and sensitive strains show the same sensitivity response to all three DMI fungicides respectively. A minority of the progeny isolates showed intermediate phenotypes in some fungicides (EC_{50} thresholds between resistant >1 mg.L^{-1}, Intermediates from 0.2 – 1 mg.L^{-1} and sensitive ≤0.2.mg.L^{-1}).The Bin range was based on the lower and upper intervals of the standard error of the difference of the ^{2}Log means. The EC_{50} positions of the parental strains are marked with triangles. Least Significant Difference (LSD) values are shown above the histograms.

From population N5, 41 markers were selected with eight markers in coupling phase with resistance and 33 markers in coupling phase with sensitivity to the tested DMIs (Figure S4b and Table S1). The markers were clustered in three groups by their genetic distance, of which 27 placed on scaffold 7. All recombination events for the N5 population are shown in the left panel of Figure 2. From the 41 markers, 32 fully co-segregate with sensitivity to the tested DMIs. Markers 12397726 and 12399875 were identified as the flanking markers with positions scaffold_7:1,879,787 bp and scaffold_7:2,175,183 bp, respectively, and a physical distance of 295,396 bp.

The order of the genetic markers in both the N2 and N5 populations was in full agreement (Figure 2). The N2 and N5 populations share 38 markers and 17 markers from both maps show inconsistencies or low coverage scores, which were therefore omitted from place them in the reference physical map. Markers with inconsistences between the physical and the genetic maps are markers 12412057 on scaffold_7 - but in a displace position - 12,412,405 on scaffold_27, marker 12397704 on scaffold_6 and marker 12410210 on scaffold_5. Since they co-segregated with the groups of markers close to or in the area carrying the sensitivity locus, we assume there are either differences between the sequences of the CIRAD86 reference genome and the parental/progeny isolates or there are a few errors in their positioning on the physical map. The positions of all markers are indicated in Figure 2 and a summary of the information is compiled in Table S1 and Figure S4. Based on the flanking markers, there is an overlapping region for the N2 and N5 populations of 250,660 bp between scaffold_7:1,879,787 (marker 12397726) and scaffold_7:2,130,447 (marker 12405280). This genetic window harbours 53 putative genes among which is *Pfcyp51*, located at scaffold_7:2,119,919-2,121,685 (Figure 2, Figure S5, Table S3).

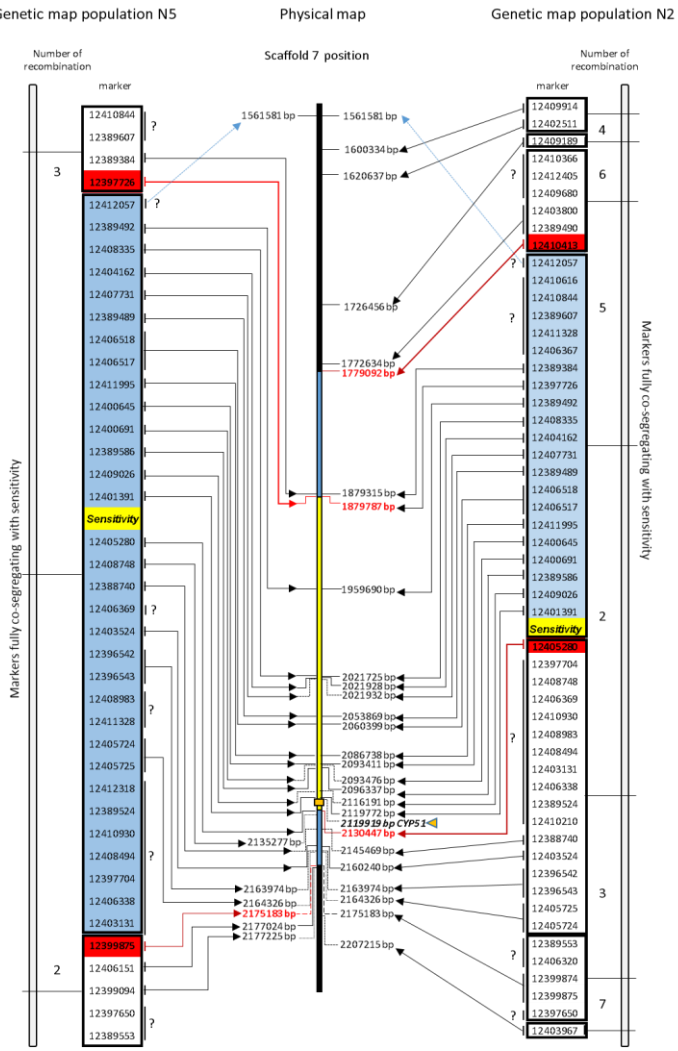

Figure 2. Integration of the *Pseudocercospora fijiensis* N2 (left) and N5 (right) genetic linkage maps with the physical map (Partial Scaffold_7) of the genomic reference *P. fijiensis* CIRAD86 (middle; *Mycosphaerella fijiensis* version 2.0). The genetic map was generated using DArTseq markers. The sensitive trait is taken as phenotypic marker in the genetic map (marker in yellow). The number of recombinations between the markers is indicated between the linkage groups. Markers perfectly co-segregating with the sensitivity trait are indicated in blue, with the direct flanking markers depicted in red for both populations. The area of the co-segregating markers for each population is presented as a light blue line and the overlapping region is depicted in yellow on the physical map. The genetic and the physical map are linked for each marker by arrows. The flanking markers are indicated in red while the remaining markers are printed in black. Genetically linked markers without a position in the physical region are indicated with a question mark (?) and unpositioned markers are placed with a light blue dashed line. The position of *Pfcyp51* is marked in bold letters with a yellow triangle and is represented by a yellow box.

Molecular analyses of the *Pfcyp51* configuration in the N2 and N5 progenies

Analysis of the *Pfcyp51* gene, including the promoter, revealed that all *P. fijiensis* progeny strains only had parental genotypes. Resistant strains carried the *Pfcyp51* gene encoding protein modifications T18I and V106D, which have no DMI phenotypic consequences, and three other substitutions related to DMI resistant describe in Figure 3, whereas all sensitive isolates were identical to the wild type genotype of the parental strain Bo_1, lacking any insertion in the promoter region and a *Pfcyp51* sequence encoding the non-phenotypical T18I, V106D and A446S amino acid (aa) modifications compared with the reference sequence of *P. fijiensis* CIRAD86 (Table S2). However, all resistant progenies from either population contained a 103 bp promoter insertion located 94 bp upstream of the reference *Pfcyp51* start codon. The insertion is accompanied with an 18 bp substitution "GGACCACTCGAACATCAC". (reference position MYCFIscaffold_7:2121783, *Mycosphaerella fijiensis* v2.0, JGI) and is composed of repeated elements interspersed with non-repeated sequences. The repeated element is described in Chong et al. (2016) (Chong *et al*. 2016b) and possesses a palindromic core. In total, three exact copies and a single modified copy of this element – from here identified as element A - are present in the insertion. The modified copy (A*) carries an additional one bp substitution, resulting in the sequence "T**A**AAATCTCGTACGATAGCA. All sensitive isolates have only one A element in their promoter (Chong et al., 2016; Figure 2). So, when taking into account this single A element in wt Bo_1, resistant progeny isolates contained five A copies (Figure 3 and Figure S3).

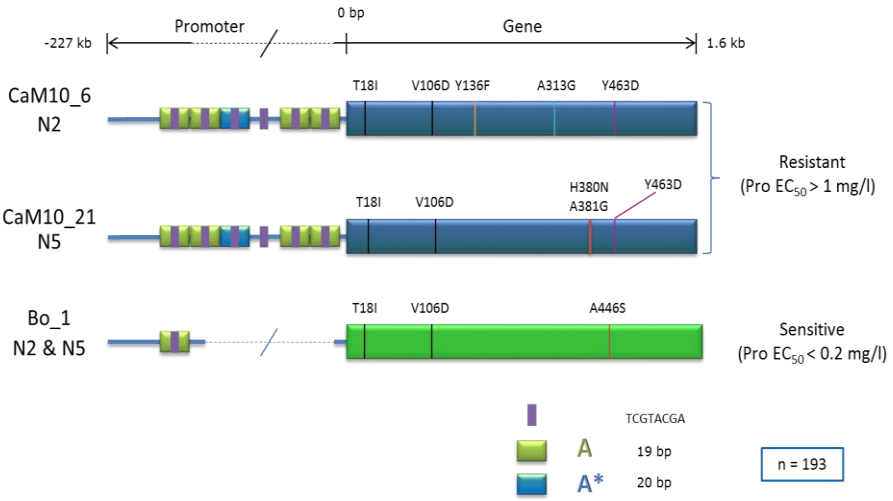

Figure 3. Schematic visualization of the *Pfcyp51* gene configuration derived from the *Pseudocercospora fijiensis* crossing partners and segregating progenies, based on the expressed phenotypes towards three DMI fungicides. All progeny isolates exclusively showed parental genotypes. Resistant isolates have promoter insertions while sensitive isolates have no insertions. Mutations in the coding domain are marked with colored lines and the resulting aa substitutions.

All resistant progeny isolates from the N2 population share *Pfcyp51* substitutions resulting in the aa modifications Y136F, A313G and Y463D originating from their resistant parent CaM10_6 (Figure 2 and Table S2). The resistant progeny from the N5 population have substitutions resulting in the aa changes H380N, A381G and Y463D, originating from the parental resistant strain CaM10_21 (Figure 3 and Table S2). All *Pfcyp51* sequences from parents and progenies of both the N2 and N5 populations showed a perfect match with the segregating phenotypes (sensitive: resistant = 1:1, Table S2). From the aforementioned four strains with intermediate behaviour, strains N2_21, N2_89 and N5_57 contained the *Pfcyp51* sequence of the sensitive parent and isolate N5_1 had the configuration of the resistant parent. Hence, their phenotypes were either scoring errors or caused by other genomic modifications.

Discussion

Disease management in agricultural crops largely depends of two crucial factors: host resistance and crop protection agents. As Cavendish bananas are highly susceptible to *P. fijiensis* and represent 85% of the global trade, there is essentially one option left for disease control, i.e. fungicides. This has huge implications for overall management. One important implication is for the disease, with an imminent risk for the selection of increasingly resistant *P. fijiensis* populations. Another important implication is on the occupational health of thousands of workers in banana plantations and the environmental issues due to the precarious tropical landscapes where bananas are usually produced (Risède *et al.* 2010). The latter issue results in significant water contaminations (van Wendel de Joode *et al.* 2016) as well as the risk of non-target hits, which for instance is considered to be the reasons of increasing fungicide resistance in *Aspergillus fumigatus* to medical azoles (Chowdhary *et al.* 2013). On top of that, the increasing loss of DMI efficacy resulted in higher frequency of contact fungicide that are more hazardous to the environment (Chong *et al.* 2016a; Guzmán *et al.* 2013; Pereira *et al.* 2014; van Wendel de Joode *et al.* 2016). Hence, a thorough analysis of DMI resistance in *P. fijiensis* is both necessary and urgent to raise awareness of spending more efforts to develop new banana germplasm with resistance to black Sigatoka (Chong *et al.* 2016a; Guzmán *et al.* 2013; Risède *et al.* 2010; Stergiopoulos *et al.* 2014; Stergiopoulos *et al.* 2010). However, this will take considerable time and hence altered strategies for the use of fungicides are required and necessitate scrutinizing the current disease control practice and its consequences. Without doubt, black Sigatoka disease is the most costly disease of global banana production with an estimated cost of at least US $\$1000.ha^{-1}$ in most environments (Arias *et al.* 2003). After describing the global landscape of DMI resistance in *P. fijiensis* (Chong *et al.* 2016b) as well as the mechanistic proof of its mechanism (Díaz-Trujillo *et al.* 2016a), we decided to perform an unbiased genetic analysis to identify any other underlying factors for the resistance to DMIs

in *P. fijiensis.* Therefore, we generated two new high marker density genetic linkage maps after crossing sensitive and resistant *P. fijiensis* isolates. These crosses were not at all routine, required a pragmatic approach compared to previous reports (Manzo-Sanchez *et al.* 2008), and eventually resulted in two mapping populations. This classic genetic approach in combination with state of the art DArTseq molecular markers technology provided novel and key information to understand the development of DMI resistance and, hence, is the basis for optimizing their use for disease management.

***Pseudocercospora fijiensis* mating**

One of the most challenging tasks in the present study was to perform the *P. fijiensis* crosses. First we were not able to unequivocally determine the mating type of each isolate, which previously also appeared difficult (Arzanlou *et al.* 2010), despite the fact that we earlier cloned the *P. fijiensis mat* genes (Conde-Ferraez *et al.* 2007). Our pragmatic approach is similar to the protocol being used for the related dothideomycete wheat pathogen *Zymoseptoria tritici* (Goodwin *et al.* 2011; Kema *et al.* 1996; Wittenberg *et al.* 2009). Recent genome data show that sub-culturing fungal isolates frequently results in chromosome loss (Johnson *et al.* 2001; Rodríguez *et al.* 2006), and hence it is conceivable that our failures to successfully cross *P. fijiensis* is related to the number of sub-cultivations of each candidate parent (Saleh *et al.* 2012). The moment we reduced these to maximally two – and essentially determined the DMI phenotype of the parents in retrospect – crosses proved to be successful, resulting in viable and sufficient progeny strains for formal genetic analyses. Hence, we conclude that subsequent sub-cultivation steps affect sexual fitness, although we do not know whether this has to do with the loss of essential chromosomes. Alteration of fungal properties by sub-cultivation not only affects mating but also for instance pathogenicity observed by Krokene and Solheim in the blue-stain fungus *Ceratocystis polonica* (Krokene & Solheim 2001) and by Kashino et al. in

Paracoccidioides brasiliensis (Kashino *et al.* 1990). Krokene and Solheim also observed alterations in the ability to grow under oxygen-deficient and reduction in growth rate in *C. polonica* (Krokene & Solheim 2001).

Sensitivity tests results

Despite the bimodal distribution into sensitive and resistant progeny, the wide range of EC_{50} values' in each group was substantial and awaits further explanation as various strains exceeded the thresholds that were recently established for sensitivity based on global population analyses (Chong *et al.* 2016b). We, therefore, adopted another threshold in this experiment as it is conceivable that the chosen fungicide doses limited the precise determination of EC_{50} concentrations, especially at low concentrations. Potentially, other individual factors such as minor fungicide resistance genes, or genes related to stress responses or growth rates are involved. Nonetheless, the DMI response difference between sensitive and resistant isolates was clear and separates them into two major and discrete groups with an approximate differential resistance factor of approximately 100 (Table 2), resulting in a 1:1 segregation, indicating a single causal gene for DMI sensitivity in *P. fijiensis.*

Genetic linkage maps

By using the DMI phenotypes and progeny genotypes in a mapping approach we show that azole sensitivity in the two segregating *P. fijiensis* populations is due to a single major gene, *Pfcyp51*. Since no evidence was observed for the presence of any other sensitive genetic region in either progeny, this strongly supports the presence of the *Pfcyp51* gene as the single explanatory factor for DMI sensitivity. The progeny was no different when compared to the parents, despite the quantitative expression of DMI sensitivity (Cañas *et al.* 2009; Chong *et al.* 2016b; Dyer *et al.* 2000). In only four progeny isolates an alternative explanation seems

appropriate, but was not apparent from the generated data set and might equally be due to experimental error due to e.g. age or density of *P. fijiensis* cultures.

The genetic window explaining DMI sensitivity contained 53 genes, of which the majority lack any functional clue. Predicted gene Id96804 encodes a putative transcription factor, which might regulate expression of (minor) genes that contribute to DMI resistance and predicted gene Id86816 encodes a putative transporter that might facilitate increased efflux (Stergiopoulos *et al.* 2002; Zwiers 2002) (Table S3). However, the overruling factor seems to be *Pfcyp51* that also maps to this exact region (Figure 2 and S4) and which accords with its importance in other – related – fungi (Cools *et al.* 2013) as well as with the accumulating evidence that recently became available (Cañas *et al.* 2009; Chong *et al.* 2010; Chong *et al.* 2016b; Díaz-Trujillo *et al.* 2016a). Therefore, despite the presence of other genes in the mapped genomic region, we propose that modifications of the *Pfcyp51* gene and its promoter are the driving molecular force for DMI fungicide resistance.

Molecular analysis of the *Pfcyp51* configuration in F1 progenies

Common mechanisms described for the loss of sensitivity to DMIs are increased efflux of the fungicides from the cells (Cools *et al.* 2013; Leroux *et al.* 2010), adaptation and overexpression of the fungicide target gene (Bolton *et al.* 2016; Chong *et al.* 2016b; Cools *et al.* 2012; Díaz-Trujillo *et al.* 2016a; Villani *et al.* 2016) and cellular alterations that reduce the toxicity of the fungicides, such as modulation of the targeted biosynthesis pathway (Cowen 2008). Recently, we showed that modulation of the promoter and coding domain of *Pfcyp51* explains reduced DMI sensitivity (Chong *et al.* 2016b; Díaz-Trujillo *et al.* 2016a). Other fungal species, such as *Candida albicans* and *Aspergillus graminearum*, accommodate more than one of these mechanisms (Akins & Sobel 2009; Becher & Wirsel 2012; Cowen 2008). Our data suggests that in *P. fijiensis* modification and consistent alteration in the promoter region of the

Pfcyp51 are the most plausible causes of the reduced sensitivity to DMIs. The current study underscores these observation as our unbiased linkage approach did not map any additional contributing factor to DMI sensitivity; none of the sensitive strains contained substitutions Y136F, A313G, H380N, A381G or Y463D or insertions in the promoter region of *Pfcyp51*, which were previously correlated with reduced efficacy in DMIs (Cañas *et al.* 2009; Chong *et al.* 2016b; Díaz-Trujillo *et al.* 2016a). Since there were no recombination events close to the *Pfcyp51* gene, we also conclude that sexual reproduction apparently does not contribute to the important *Pfcyp51* promoter modulations, which therefore awaits further mechanistic explanations.

Interestingly, the overall sensitivity loss in population N2 was higher than in N5. Based on the similarity of the promoter configuration we assume that the expression of the gene is comparable (Table 2). Therefore, the sensitivity difference might be explained by the non-synonymous mutations present in the *Pfcyp51* gene (Figure 2). Resistant progeny from N2 harbour aa substitutions Y136F, A313G and Y463D, while those in N5 comprise H380N, A381G and Y463D. The differentiating substitutions at positions 136 and 313 are particularly important since these are located in the substrate binding site (Cañas *et al.* 2009; Chong *et al.* 2016b).

Unlike other single site fungicide interactions, CYP51 substitutions often affect individual, or a subset of DMIs compounds, with generally incomplete cross-resistance across the whole class (Cools et al., 2013). This particular mode of interaction of the CYP51 protein can explain the unusual behaviour and the steep increase of DMI resistance in the banana plantations. It is likely that the quantitative resistance response – as observed for DMI resistance in the field - is due to an accumulation of different *Pfcyp51* non-synonymous mutations in response to the apparent selection pressure, which also and alternatively can be due to a

dramatic synergistic and epistatic effect explained by *Pfcyp51* overexpression under high selection pressure (Díaz-Trujillo *et al.* 2016a).

In comparison with the DMI resistance mechanisms present in other fungi (Becher & Wirsel 2012; Cools *et al.* 2013) it is at least remarkable that we have exclusively identified a role for *Pfcyp51* in DMI sensitivity. The lack of alternative quantitative mechanisms might indicate that we are just at the beginning of *P. fijiensis* DMI resistance development, illustrative for the recent development in DMI resistance in Latin America. Common DMI resistance mechanisms in other fungi include increased fungicide efflux or alternative ergosterol synthesis pathways (Cools *et al.* 2013; Cowen 2008) may therefore become more common in the future if the fitness penalty for these mechanisms is sufficiently low (Cools *et al.* 2013; Cowen 2008). However, this largely depends on future application of DMI fungicides. The current situation on reduced DMI efficacy has already resulted in a fall-back strategy avoiding DMIs and increased use of protectants and mineral oil (Chong *et al.* 2016a).

Our data have not shown any modulating effect of sexual reproduction on *Pfcyp51*, but the versatility of the fungus through an almost continuous production of offspring throughout the year is undeniable. Current spraying practices likely significantly contributed to the accumulation and actually fixation of strobilurin resistance, as was recently explained in *Zymoseptoria tritici* (Aouini 2016). This should - evidently - be a huge concern for the banana industry since the maximum number of fungicide applications seems to have plateaued and thus the efficacy of the treatments could be on the verge of breaking. In other species there are few indications that a temporal suspension of DMI applications results in a subsequent decrease of resistant isolates due to fitness penalty and stability restrains in the *Pfcyp51* gene (Chowdhary *et al.* 2013; Lendenmann *et al.* 2015; Verweij *et al.* 2013), although in a limited number of cases this strategy was shown to be functional (Latin, 2011, Cowen, 2008). As stated above, resistance breeding, but also the introduction of biological control measures and the

development of alternative non-DMI fungicides are considered as the most promising options for a more balanced and hence sustainable black Sigatoka management.

Acknowledgements

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Supporting information

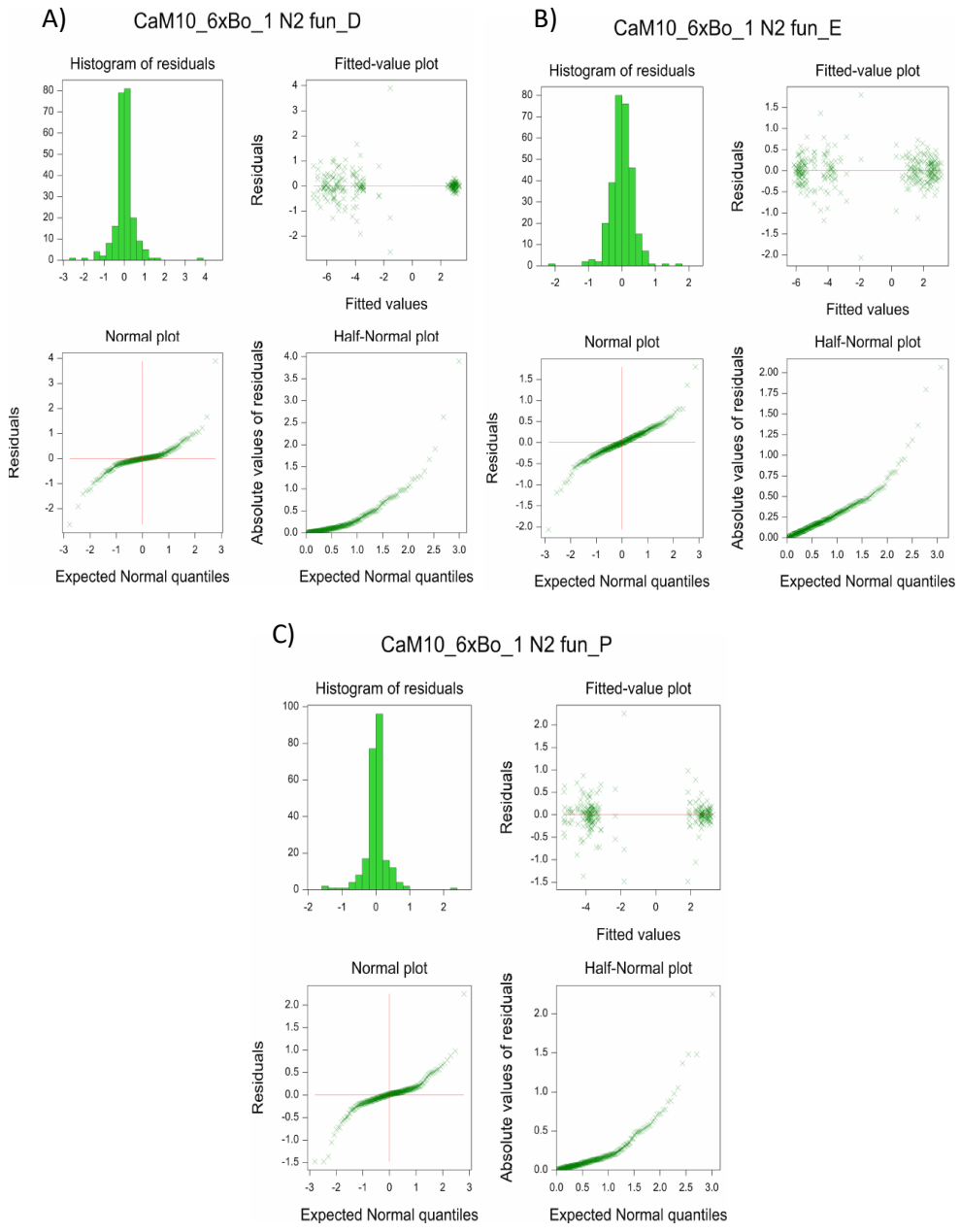

Figure S1. Plots of the calculated EC_{50} values and residuals of the interaction of the *Pseudocercospora fijiensis* segregating N2 population with the three DMI fungicides. A) difenoconazole, B) epoxiconazole and C) propiconazole.

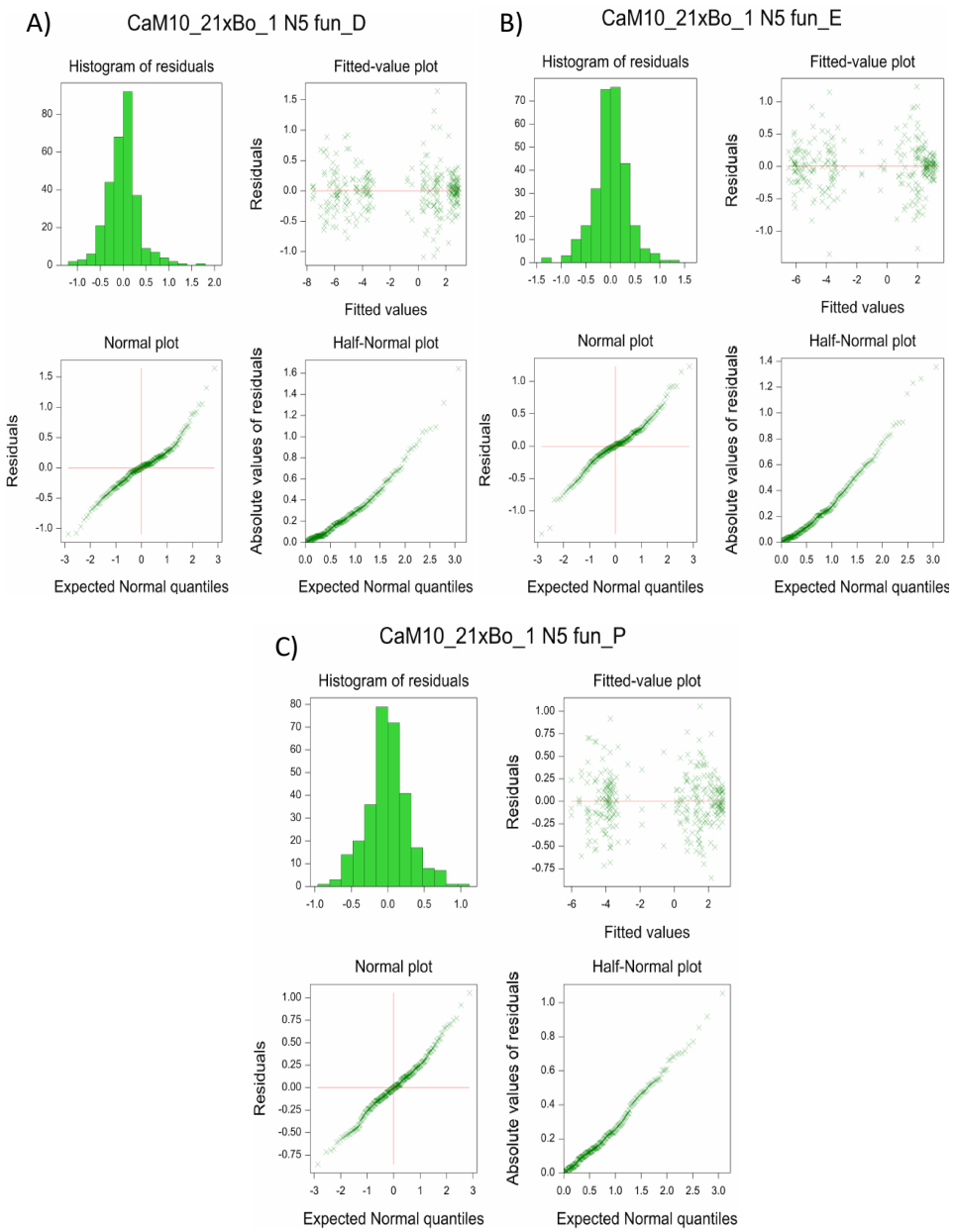

Figure S2. Plots of the calculated EC_{50} values and residuals of the interaction of the *Pseudocercospora fijiensis* segregating N5 population with the three DMI fungicides. A) difenoconazole, B) epoxiconazole and C) propiconazole

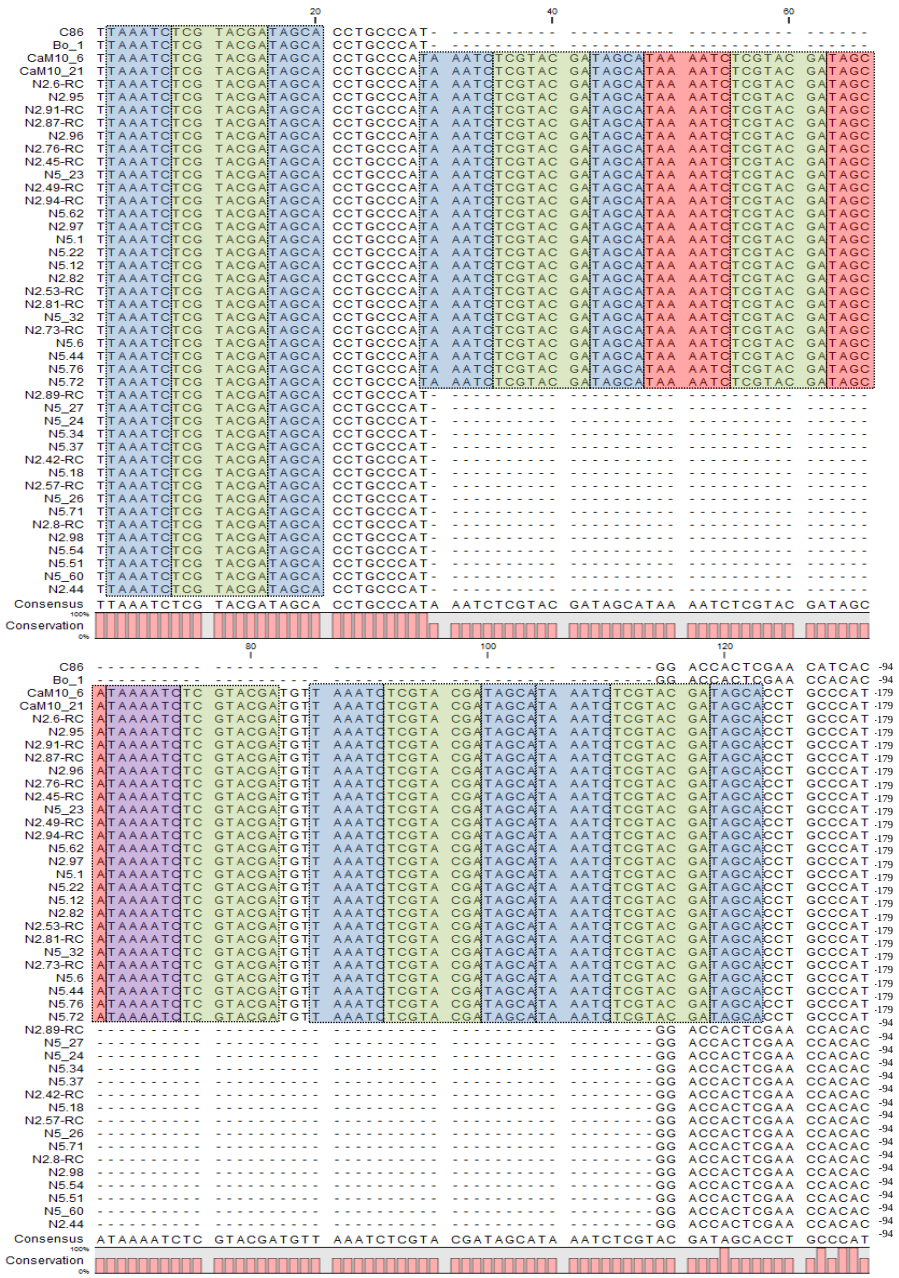

Figure S3. Analysis of the insertions in the promoter region of *Pfcyp51* gene in *Pseudocercospora fijiensis* progeny strains in both the N2 and N5 mapping populations. The promoter modifications start at -94 bp upstream of the *Pfcyp51* start codon of the reference sequence. Element "A" is shown in blue boxes together with the arrangement of the palindromic sequence TCGTACGA shown in green boxes. Element "A*" is shown in red as a partial construction of element "A" in purple. Negative values in the right bottom represent the positions from the beginning of the insertion related to the start codon of the gene.

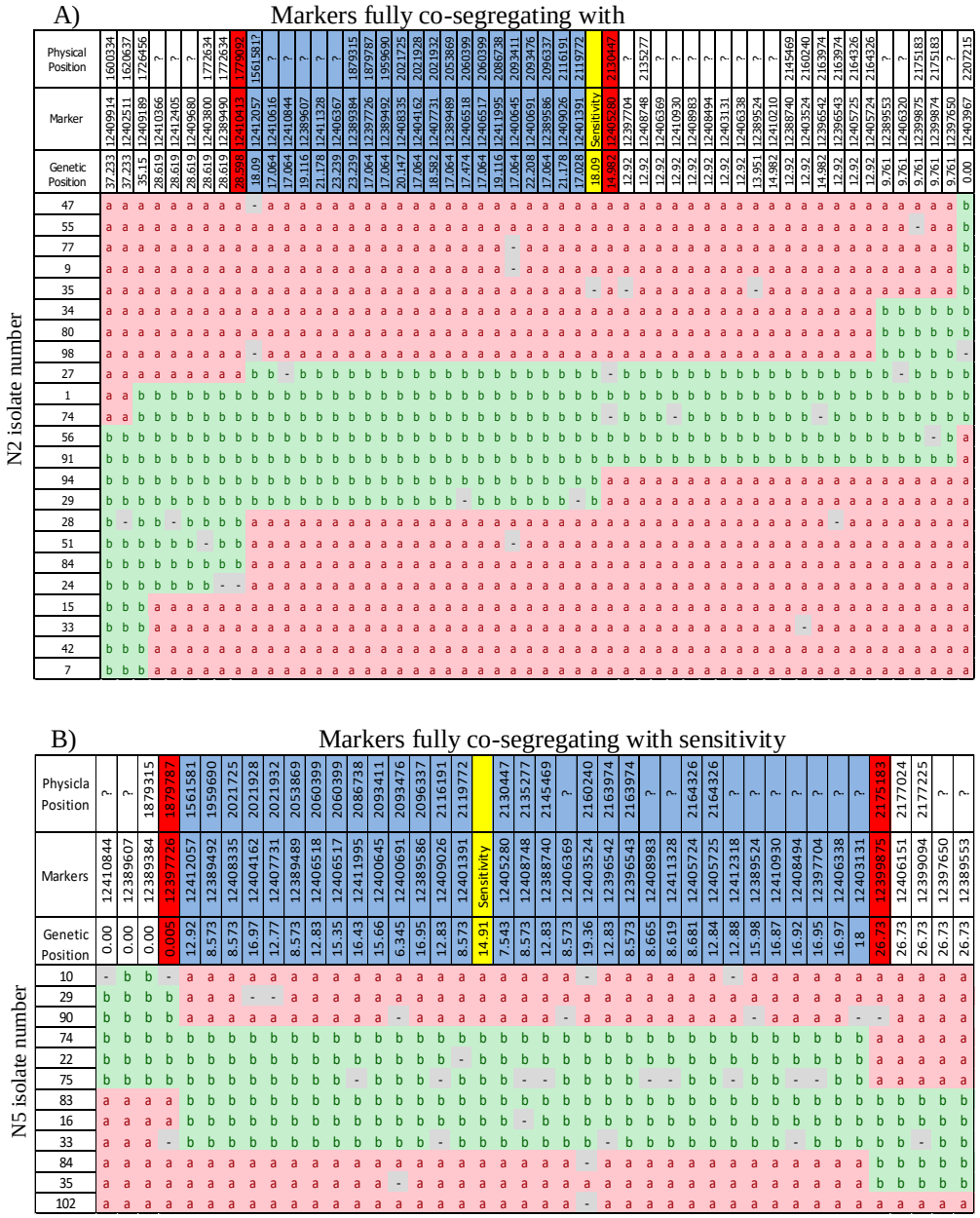

Figure S4. *Pseudocercospora fijiensis* strains sorted according to the positions of the recombination events in the chromosomal region harbouring the *Pfcyp51* sensitivity gene. A) Mapping population N2 and B) Mapping population N5. The markers descending from the highly sensitive parent are coded "a" and shown in light red boxes, whereas the chromosomal segments inherited from the resistant parent genotype are coded "b" and shown in light green boxes. The unknown values are represented by dashes in grey boxes (-). The DArTseq markers fully co-segregating with sensitivity are shown in light blue with the sensitivity trait shown in yellow. The flanking markers are shown in red.

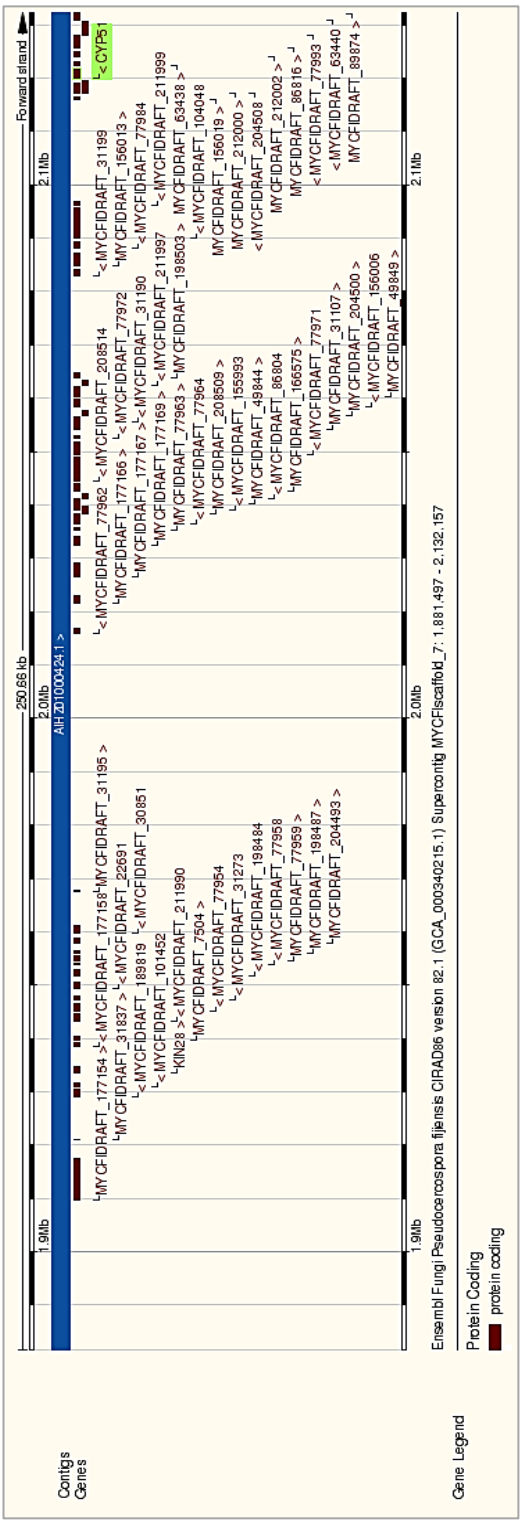

Figure S5. Scheme of the position of the 53 putative genes found in the overlapping genetic window of the *Pseudocercospora fijiensis* N2 and N5 mapping populations that contains the sensitivity region. The *cyp51* gene is highlighted in green. The figure was taken from the Ensemble fungi portal website, http://fungi.ensembl.org/Pseudocercospora_fijiensis_cirad86/Location/View?r=MYCFIscaffold_7:1879787-2130447;site=ensemblthis;db=core.

Table S1. Sequence information of the DArTseq markers used to generate the *Pseudocercospora fijiensis* N2 and N5 population linkage maps. The markers were selected based on their co-segregation with the sensitivity trait.

N2	N5	Genome Reference %ID	coverage score	Coverage %	Scaffold best hit	Reference Genome Position		Marker Sequence	N2 sensitivity segregation score		N5 sensitivity segregation score	
Marker ID code						Beginning	End		Sensitive	Resistant	Sensitive	Resistant
12409914	-	100	68.00	98.55	7	1600334	1600402	TGCAGATTTATGATGGGTGGCTCATTCGGCCTTTG GTTGGGAAGCAGATTGAGCAGCGAGAGCGAGTTT	0.195	0.918	-	-
12402511	-	100	68.00	98.55	7	1620637	1620705	TGCAGTTCGACTTCTTCGAGTTCAATGCCATCTGT GCTGCGAATGCGCCAGTTCTCCTTCCGCTTGGCA	0.167	0.913	-	-
12409189	-	100	35.00	50.72	7	1726456	1726491	TGCAGCTGGAGAGGAAGTCTGGTCGAACCATTCTT ACAGATCGGAAGAGCGGTTCAGCAGGAATGCCGA	0.205	0.940	-	-
12410366	-	many hits	?	?	?	?	?	TGCAGTAGCAAATTTGCTACTGCCTAGGCGCCCTA AGGAGTACTCTAATCGATGCTAGACGGGACTAGT	0.091	0.960	-	-
12412405	-	100	65.00	94.20	27(?)	6204	6269	TGCAGCCTATAGAATTCGTATACAACAATAGTGT ATACTTATCTACTAAGGTTTTACTATATATAGCTA	0.070	0.955	-	-
12409680	-	100	65.00	94.20	1	371382	371447	TGCAGCAGGCAAATGCTTATACCTAGGCTGCTAC TTAGGGACGTAGTCGTAGTCTAGGTTCTAGGTTGT	0.091	0.960	-	-
12403800	-	100	68.00	98.55	7	1772634	1772702	TGCAGGACGCGTGCCAAGCCAGAGTGGGGCCTGC TTGACTTCGACTTTTGAGGAGCTCTGGCCAAACGA	0.070	0.959	-	-
12389490	-	100	47.00	68.11	7	1772634	1772681	TGCAGGACGCGTGCCAAGCCAGAGTGGGGCCTGC TTGACTTCGACTTTGAGGAGCTCTGGCCAAACGAT	0.927	0.040	-	-
12410413	-	100	53.00	76.81	7	1779092	1779145	TGCAGCGTTCTCAACAGGGTCCTGATGGCAGGAGG AACGAGAGGAGGGTTATTACAGATCGGAAGAGCG	0.929	0.043	-	-
12410616	-	two hits	41	59.42	9 & 17(?)	?	?	TGCAGTATCTTCTAACTAGACAAGACCTAACATT ACTTATACTGTTACAGATCGGAAGAGCGGTTCAG	1.000	0.022	-	-
12410844	12410844	many hits	?	?	?	?	?	TGCAGCTGCTAGAAAAGATAATAGCAGCGATGAT AATAACGAGTGTAAATCAGATTCTAACTCGGAAGG	1.000	0.022	0.947	0.078
12389607	12389607	many hits	?	?	?	?	?	TGCAGTAGTAGTAATAGCGCAGTTAGAAGTTAGA TATTGTATTAGAGGTAAGGTTTACAGATCGGAAGA	1.000	0.021	0.919	0.080
12406367	-	many hits	Low	?	?	?	?	TGCAGTAACAATAAGGTGTTATATAGCTATGTAAA TTACAGATCGGAAGAGCGGTTCAGCAGGAATGCC	1.000	0.021	-	-
12389384	12389384	100	65.00	94.20	7	1879315	1879380	TGCAGAGGTAGAATTCTTCCATTGCCTTCTCGCAA TGCAAGGATATGCGCAAATGTAACATACATACAT	1.000	0.021	0.925	0.078
12397726	12397726	100	65.00	94.20	7	1879787	1879852	TGCAGGAGTATTACTTCGCGAAGCACCGGGTAGC TTCGATGAGGTTGCTAGGCCGTCTAGAACCCGCGT	1.000	0.020	0.947	0.060
12412057	12412057	100	68.00	98.55	7(?)	1561581	1561649	TGCAGGTTTTCTTTTAGTTCCTAGGTGTTTTCTAA ATATAGGTATCCTTTCTATTTGATAAGGTATTTC	0.000	1.000	0.000	0.961
12411328	12411328	many hits	Low	?	?	?	?	TGCAGTTTGAAATATTCTACAATGCCATAGTTCTC ATCGGCCATGCAATTACAGATCGGAAGAGCGGTT	1.000	0.021	1.000	0.021
12389492	12389492	100	68.00	98.55	7	1959690	1959758	TGCAGGAGAATCTTCCAGGCCATCAGGCTAGGGT CTTCGCCGTTGATTGGTCGCCTGATGGAGAGCGTG	1.000	0.021	1.000	0.060
12408335	12408335	100	68.00	98.55	7	2021725	2021793	TGCAGCTCCCTCGACCGTAGTTCTAGTTATCTGTA CAGAGCGGAGAATCTCTCGAGTCAGCGGTCGCCA	0.976	0.021	1.000	0.021
12404162	12404162	100	68.00	98.55	7	2021928	2021996	TGCAGCAGTTTTCCTGAACCGGGCTTCCGATGTGC AGGCTTTCATGGAGCTCGGAGCTAATGCTTCTGG	1.000	0.000	1.000	0.020
12407731	12407731	100	67.00	97.10	7	2021932	2021999	TGCAGTTTTCCTGAACCGGGCTTCCGATATGCAGG CTTTCATGGAGCTCGGAGCTAATGCTTCTGGCCG	0.000	1.000	0.000	0.979

N2	N5	Genome Reference	coverage score	Coverage %	Scaffold best hit	Reference Genome Position		Marker Sequence	N2 sensitivity segregation score		N5 sensitivity segregation score	
Marker ID code		%ID				Beginning	End		Sensitive	Resistant	Sensitive	Resistant
12389489	12389489	100	68.00	98.55	7	2053869	2053937	TGCAGGAATGTGGCAGTTTCGGCTTAGAAATGCA AGGAGGATTGTGTTTTCAGTCCGGCCGATCTTGTC	1.000	0.020	1.000	0.039
12406518	12406518	100	68.00	98.55	7	2060399	2060467	TGCAGAGCTGTCATCTCCTACCGACGGATGTGTCT GGGAAAGGCGGTCAATGCTGGTGCATATGGCCAT	0.000	1.000	0.000	0.960
12406517	12406517	100	68.00	98.55	7	2060399	2060467	TGCAGCGCTGTCATCTCCTACCAACGGATGTGTCT GGGAAAGGCGGTCAATGCTGGTGCATATGGCCAT	1.000	0.021	1.000	0.020
12411995	12411995	100	68.00	98.55	7	2086738	2086806	TGCAGAGGAGCTCGCACTCAAGCGGCAAGCACAT GAGCAGATACGCAAGTGCATGGGCAAGATCTCGAA	1.000	0.021	1.000	0.021
12400645	12400645	100	68.00	98.55	7	2093411	2093479	TGCAGCGCACGCATTTGGCATCCTGGCTTCGCATC CAAGCGCAAGAGCCCAGCAGCAACTTGCACAGCT	1.000	0.020	1.000	0.000
12400691	12400691	100	68.00	98.55	7	2093476	2093544	TGCAGTCATGCGACGACCATGGTGATTGGATTTGA GTAAGGTTAGGAGAAACTCGGTCCGTCCAACGTG	0.976	0.020	1.000	0.038
12389586	12389586	100	68.00	98.55	7	2096337	2096405	TGCAGTTTGAGGGGAAGGATTGGTTTGCTTGCCCA GTCGATGAATTCAACACTGGGTACAGCATCTGGG	1.000	0.020	1.000	0.020
12409026	12409026	100	68.00	98.55	7	2116191	2116259	TGCAGACTCCGACCAAGGCTAGAGTTATCACATAG TAAACTAAATGGATCTGCGTGAATGGCTGCAAGT	0.000	1.000	0.000	0.956
12401391	12401391	100	44.00	63.76	7	2119772	2119816	TGCAGCGCACTACCAACGCAGCCACAGGCATCCC TTTGTCTCGGTTACAGATCGGAAGAGCGGTTCAGC	1.000	0.000	1.000	0.020
12405280	12405280	100	68.00	98.55	7	2130447	2130515	TGCAGCTCGGTGGCTTCGTGGACGCACTTGAGATG TTATGCTGCGGTTGAGTGAGAAGATATCAGAAGC	1.000	0.065	0.974	0.060
12397704	12397704	100	57.00	82.60	6 (?)	4164497	4164554	TGCAGCTAAGAAGCCTACCGCCCCGAAGAACAAT ACACTAGCTGCACCTAAGGTAGCTACTAGCAATAA	1.000	0.061	1.000	0.020
12408748	12408748	100	45.00	65.21	7	2135277	2135321	TGCAGTCAAGCAAGTGCAGACTTGGTCCATGGAAA GAGCGGGTCTTACAGATCGGAAGAGCGGTTCAGC	1.000	0.063	1.000	0.022
12406369	12406369	No hit	?	?	?	?	?	TGCAGTAGTAATAGCATTCAATTCAATATCTACTA TATTACAGATCGGAAGAGCGGTTCAGCAGGAATG	1.000	0.061	1.000	0.058
12410930	12410930	many hits	?	?	?	?	?	TGCAGGAATACGCAGCTCTCCTAGACGTCGGTAG CTATTCCTAAAATTGCACTTAGGACAGCTCTTATA	1.000	0.064	1.000	0.039
12408983	12408983	many hits	?	?	?	?	?	TGCAGTAGTGCCTGTTGCGACGGGTATAGGCGTCT TAGTCGTATTATTCTTACAGATCGGAAGAGCGGT	1.000	0.061	1.000	0.021
12408494	12408494	many hits	Low score	?	?	?	?	TGCAGACCGGCCCGCTAGCGGCGCTATTACAGATC GGAAGAGCGGTTCAGCAGGAATGCCGAGACCGAT	1.000	0.061	1.000	0.021
12403131	12403131	many hits	Low score	?	?	?	?	TGCAGTACTTTATAACAACGAAACAGCTTACAGAT CGGAAGAGCGGTTCAGCAGGAATGCCGAGACCGA	1.000	0.061	0.974	0.058
12406338	12406338	two hits	Low score	?	1(?)	?	?	TGCAGTAAACCCGCCTACTTTTACAGATCGGAAGA GCGGTTCAGCAGGAATGCCGAGACCGATCTCGTA	1.000	0.061	1.000	0.059
12389524	12389524	many hits	Low score	?	?	?	?	TGCAGGTAGTAGTAGTGGTTTTGGTTTCCGTCCTT TAGTAGGTAGATAGGGTAGTAGTAAATTAGTATA	0.974	0.060	1.000	0.020
12410210	-	100	66.00	95.65	5 (?)	513760	513826	TGCAGCCGGGAACGCGTACGGAGGGGCACCGTAC TTGTTTGTAAGACTAGAGGGCGAGGTAGGGCTAGG	1.000	0.086	-	-
-	12412318	many hits	?	?	?	?	?	TGCAGAACTGTTAGTAGATAATCAAAAAATTTCAA AACCGAATAAAAAAGAAATAACTAGTATAGGAAT	-	-	0.000	0.955
12388740	12388740	100	68.00	98.55	7	2145469	2145537	TGCAGAACACTCCTGGGAACTCACATCTCAGCAT CGTTGTTTTGCTGCGTAGAGAAACATGGGATGCGC	0.000	0.956	0.000	0.929

N2	N5	Genome Reference	coverage score	Coverage %	Scaffold best hit	Reference Genome Position		Marker Sequence	N2 sensitivity segregation score		N5 sensitivity segregation score	
Marker ID code		%ID				Beginning	End		Sensitive	Resistant	Sensitive	Resistant
12403524	12403524	100	68.00	98.55	7	2160240	2160308	TGCAGAGTCAATCATAGTCGCGAGGAAAGCACAC GAGAGGTAGATGTAGGTAATGATGCTCTGCTCTGC	1.000	0.060	0.967	0.057
12396542	12396542	100	68.00	98.55	7	2163974	2164042	TGCAGAAACTCAGAGTCATGCTTTTGGGCCGTCG CCCTGGTTTTCATCAAGAGAGAACATTATACAATC	0.000	0.951	0.000	0.957
12396543	12396543	100	68.00	98.55	7	2163974	2164042	TGCAGAAGCTCAGAGTCATGCTTTTGGGCCGTCG CCCTGGTTTTCATCAAGAGAGAACATTATATAATC	1.000	0.060	1.000	0.038
12405725	12405725	100	68.00	98.55	7	2164326	2164394	TGCAGAATCGAGACTTCTTTGCTTGACTCTACTTC CTCAGGCGAGACAGCCGTGTTCGTGGCAAGCAAA	0.000	0.959	0.000	0.957
12405724	12405724	100	68.00	98.55	7	2164326	2164394	TGCAGAATCGAGACTTCTTTGCTTGACTCGACTTC CTCAGGCGAGACGGCCGTGTTCGTGGCAAGCAAA	1.000	0.065	1.000	0.020
12399874	-	100	68.00	98.55	7	2175183	2175251	TGCAGCCCAACGCTCCGCCACCAGAGCAGGTTCGA GCTCGGCAGGAGCAGGATCGGCAGCGTGAGCAGG	0.068	0.957	-	-
12399875	12399875	100	68.00	98.55	7	2175183	2175251	TGCAGCCCAACGTTCCGCCACCAGATCAGGTTCGA GCTCGGCAGGAGCAGGATCGGCAGCGTGAGCAGG	0.925	0.060	0.947	0.096
12406320	-	many hits	Low score	?	?	?	?	TGCAGCAATTTCATCCTACTTACTTAGAGGTCCTT TAGATATATTACAGATCGGAAGAGCGGTTCAGCA	0.070	0.956	-	-
12389553	12389553	many hits	Low score	?	?	?	?	TGCAGTCAGCGCAGATGGCCATCGATAAATTGATG AGCGATAGGGCGGCCGAGAAAGCAGCTTATGAGA	0.930	0.063	0.950	0.078
-	12406151	97	29.00	42.02	7	2177024	2177056	TGCAGCTCTGTCGGTCCTTGCGCTGGGCTCCGAGA CCGACGCTCGTCTCTCCGGAAGGAGCCATGGGTC	-	-	0.950	0.082
-	12399094	93.9	25.00	36.23	7	2177225	2177257	TGCAGCCTGGCGTCGAATCTCCTCTTCGGCCTTAC AGATCGGAAGAGCGGTTCAGCAGGAATGCCGAGA	-	-	0.950	0.080
12397650	12397650	many hits	Low score	?	?	?	?	TGCAGAGCTCGCAGAAGAGCGGCGGGTGTACCAT CTTGCTCTGCGCGAGATGGCGATGAGGAATCTTGC	0.930	0.064	0.950	0.082
12403967	-	100	68.00	98.55	7	2207215	2207283	TGCAGTCCTGTATGGAGCCAGCAGATGGCTTATTA CACCAATGGTAGACGCGCTATCTGAGTCGCGACA	0.140	0.909	-	-

Table S2. Sequence information of the *Pfcyp51* gene of the *Pseudocercospora fijiensis* parental isolates and the N2 and N5 progenies and their average EC_{50} scores against the three DMI fungicides difenoconazole, epoxiconazole and propiconazole. Sensitive isolates are marked with green background and resistant isolates with a red background based on their EC_{50} scores where isolates with scores <0.2 $mg.L^{-1}$ were classified as sensitive and isolates with scores >1.00 $mg.L^{-1}$ were classified as resistant. Isolates with intermediate response are marked in orange. Isolates with question marks (?) remained undetermined.

number	Isolate	Insertions in the promoter of the *cyp51* gene	Amino acid CYP51 substitutions								DMI fungicide EC_{50} average scores		
		Promoter	T18	V106	Y136	A313	H380	A381	Y463	A446	Difenoconazole	Epoxiconazole	Propiconazole
Ref_1	Bo_1	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.006	0.013	0.018
Ref_2	CaM10_6	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	17.194	7.496	11.750
1	N2_1	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	>10.24	5.036	>10.24
2	N2_2	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	9.086	6.886	5.521
3	N2_3	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	7.900	4.059	6.082
4	N2_4	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.017	0.026	0.052
5	N2_5	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	9.420	3.693	6.392
6	N2_6	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	8.313	5.966	5.958
7	N2_7	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.099	0.089	0.102
8	N2_8	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.014	0.016	0.034
9	N2_9	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.028	0.036	0.069
10	N2_10	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	8.749	4.855	>10.24
11	N2_11	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	>10.24	5.553	>10.24
12	N2_12	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	6.528	2.690	5.385
13	N2_13	?	?	?	?	?	?	?	?	?	7.994	2.320	6.199
14	N2_14	WT	T18I	V106D	WT		WT	WT	WT	A446S	0.037	0.064	0.070

number	Isolate	Insertions in the promoter of the *cyp51* gene	Amino acid CYP51 substitutions								DMI fungicide EC_{50} average scores		
		Promoter	T18	V106	Y136	A313	H380	A381	Y463	A446	Difenoconazole	Epoxiconazole	Propiconazole
15	N2_15	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.034	0.052	0.056
16	N2_16	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.017	0.025	0.057
17	N2_17	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.059	0.069	0.073
18	N2_18	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	8.474	1.995	8.059
19	N2_19	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	>10.24	4.332	>10.24
20	N2_20	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	7.132	1.894	8.337
21	N2_21	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.213	0.111	0.120
22	N2_22	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.022	0.021	0.063
23	N2_23	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	8.459	3.239	6.902
24	N2_24	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.010	0.015	0.027
25	N2_25	WT	T18I	V106D	WT		WT	WT	WT	A446S	0.094	0.086	0.081
26	N2_26	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.091	0.036	0.090
27	N2_27	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	9.589	7.057	8.697
28	N2_28	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.061	0.036	0.074
29	N2_29	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	8.274	4.781	6.005
30	N2_30	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	8.064	3.219	7.883
31	N2_31	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.032	0.024	0.080
32	N2_32	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.042	0.031	0.077
33	N2_33	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.082	0.073	0.088
34	N2_34	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.017	0.018	0.065
35	N2_35	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.099	0.180	0.135
36	N2_36	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.019	0.020	0.055
37	N2_37	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	6.654	2.838	6.327
38	N2_38	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	>10.24	>10.24	>10.24

number	Isolate	Insertions in the promoter of the *cyp51* gene	Amino acid CYP51 substitutions							DMI fungicide EC_{50} average scores		
		Promoter	T18	V106	Y136	A313	H380	A381	Y463	A446	Difenoconazole	Epoxiconazole	Propiconazole
39	N2_39	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	9.127	4.996	4.463
40	N2_40	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	9.310	3.568	9.174
41	N2_41	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.009	0.018	0.048
42	N2_42	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.013	0.018	0.036
43	N2_43	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.013	0.018	0.028
44	N2_44	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.068	0.061	0.073
45	N2_45	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	10.072	5.651	>10.24
46	N2_46	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	>10.24	5.014	>10.24
47	N2_47	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.031	0.021	0.057
48	N2_48	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	8.675	2.173	9.186
49	N2_49	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	8.196	5.808	>10.24
50	N2_50	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	10.142	2.202	10.051
51	N2_51	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.030	0.018	0.059
52	N2_52	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	>10.24	6.359	>10.24
53	N2_53	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	7.049	5.746	6.272
54	N2_54	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.068	0.056	0.082
55	N2_55	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.045	0.058	0.076
56	N2_56	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	>10.24	8.899	9.446
57	N2_57	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.091	0.066	0.101
58	N2_58	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.087	0.058	0.102
59	N2_59	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.078	0.073	0.077
60	N2_60	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	>10.24	3.645	>10.24

number	Isolate	Insertions in the promoter of the *cyp51* gene	Amino acid CYP51 substitutions							DMI fungicide EC_{50} average scores		
		Promoter	T18	V106	Y136	A313	H380	A381	Y463	A446	Difenoconazole	Epoxiconazole	Propiconazole
61	N2_61	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATTAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	8.646	6.709	7.779
62	N2_62	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATTAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	>10.24	3.105	>10.24
63	N2_63	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATTAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	9.886	2.980	7.598
64	N2_64	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.063	0.017	0.075
65	N2_657	?	?	?	?	?	?	?	?	?	7.457	1.351	3.897
66	N2_66	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATTAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	>10.24	7.392	8.710
67	N2_67	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.014	0.015	0.027
68	N2_68	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATTAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	7.430	4.047	5.682
69	N2_69	WT	T18I	V106D	WT		WT	WT	WT	A446S	0.025	0.018	0.067
70	N2_70	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATTAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	8.125	6.326	6.596
71	N2_71	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATTAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	7.181	2.295	6.488
72	N2_72	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.025	0.020	0.068
73	N2_73	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATTAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	8.169	7.973	8.452
74	N2_74	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATTAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	>10.24	7.302	8.374
75	N2_75	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATTAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	7.564	5.268	4.859
76	N2_76	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATTAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	5.669	1.612	4.982
77	N2_77	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.025	0.023	0.080
78	N2_78	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATTAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	9.560	6.697	7.277
79	N2_79	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.093	0.078	0.095
80	N2_80	WT	T18I	V106D	WT		WT	WT	WT	A446S	0.032	0.020	0.080

number	Isolate	Insertions in the promoter of the *cyp51* gene	Amino acid CYP51 substitutions							DMI fungicide EC_{50} average scores		
		Promoter	T18	V106	Y136	A313	H380	A381	Y463	A446	Difenoconazole	Epoxiconazole	Propiconazole
81	N2_81	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCACCTGCCCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	>10.24	2.780	>10.24
82	N2_82	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCACCTGCCCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	7.223	4.711	6.111
83	N2_83	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.015	0.017	0.031
84	N2_84	WT	T18I	V106D	WT		WT	WT	WT	A446S	0.023	0.027	0.072
85	N2_85	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCACCTGCCCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	7.696	2.712	8.177
86	N2_86	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.025	0.020	0.068
87	N2_87	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCACCTGCCCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	7.786	3.531	6.172
88	N2_88	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.026	0.018	0.080
89	N2_89	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.112	0.154	0.210
90	N2_90	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCACCTGCCCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	7.799	5.684	3.958
91	N2_91	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCACCTGCCCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	>10.24	5.969	>10.24
92	N2_92	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.022	0.021	0.067
93	N2_93	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.011	0.019	0.046
94	N2_94	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCACCTGCCCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	>10.24	5.477	>10.24
95	N2_95	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCACCTGCCCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	>10.24	9.276	>10.24
96	N2_96	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCACCTGCCCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	>10.24	7.419	>10.24
97	N2_97	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCACCTGCCCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	7.248	2.563	3.732
98	N2_98	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.015	0.022	0.068
99	N2_99	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCACCTGCCCAT	T18I	V106D	Y136F	A313G	WT	WT	Y463D	WT	8.764	5.704	>>10.24
100	N2_100	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.072	0.067	0.113

number	Isolate	Insertions in the promoter of the *cyp51* gene	Amino acid CYP51 substitutions							DMI fungicide EC_{50} average scores		
		Promoter	T18	V106	Y136	A313	H380	A381	Y463	A446	Difenoconazole	Epoxiconazole	Propiconazole
102	N5_2	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.079	0.142	0.157
103	N5_3	AAATCTCGTACGATAGCATAAAATCTCGTACGATAGCATAAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAA TCTCGTACGATAGCACCTGCCCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	2.104	3.162	4.616
104	N5_4	WT	T18I	V106D	WT		WT	WT	WT	A446S	0.020	0.035	0.069
105	N5_5	AAATCTCGTACGATAGCATAAAATCTCGTACGATAGCATAAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAA TCTCGTACGATAGCACCTGCCCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	2.589	5.128	2.931
106	N5_6	AAATCTCGTACGATAGCATAAAATCTCGTACGATAGCATAAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAA TCTCGTACGATAGCACCTGCCCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	5.675	6.923	6.329
107	N5_7	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.067	0.073	0.084
108	N5_8	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.011	0.015	0.031
109	N5_9	AAATCTCGTACGATAGCATAAAATCTCGTACGATAGCATAAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAA TCTCGTACGATAGCACCTGCCCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	5.140	6.041	5.197
110	N5_10	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.011	0.015	0.032
111	N5_11	AAATCTCGTACGATAGCATAAAATCTCGTACGATAGCATAAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAA TCTCGTACGATAGCACCTGCCCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	7.527	7.976	7.058
112	N5_12	AAATCTCGTACGATAGCATAAAATCTCGTACGATAGCATAAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAA TCTCGTACGATAGCACCTGCCCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	6.973	8.439	7.132
113	N5_13	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.047	0.060	0.066
114	N5_14	AAATCTCGTACGATAGCATAAAATCTCGTACGATAGCATAAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAA TCTCGTACGATAGCACCTGCCCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	1.556	1.717	1.323
115	N5_15	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.047	0.081	0.077
116	N5_16	AAATCTCGTACGATAGCATAAAATCTCGTACGATAGCATAAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAA TCTCGTACGATAGCACCTGCCCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	6.259	8.082	3.523
117	N5_17	AAATCTCGTACGATAGCATAAAATCTCGTACGATAGCATAAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAA TCTCGTACGATAGCACCTGCCCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	6.120	6.266	3.562
118	N5_18	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.010	0.011	0.032
119	N5_19	AAATCTCGTACGATAGCATAAAATCTCGTACGATAGCATAAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAA TCTCGTACGATAGCACCTGCCCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	3.355	6.146	2.787
120	N5_20	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.012	0.016	0.052
121	N5_21	AAATCTCGTACGATAGCATAAAATCTCGTACGATAGCATAAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAA TCTCGTACGATAGCACCTGCCCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	1.278	2.714	1.135
122	N5_22	AAATCTCGTACGATAGCATAAAATCTCGTACGATAGCATAAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAA TCTCGTACGATAGCACCTGCCCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	1.683	1.875	1.793

number	Isolate	Insertions in the promoter of the *cyp51* gene	Amino acid CYP51 substitutions							DMI fungicide EC_{50} average scores		
		Promoter	T18	V106	Y136	A313	H380	A381	Y463	A446	Difenoconazole	Epoxiconazole	Propiconazole
123	N5_23	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	1.658	3.687	2.518
124	N5_24	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.086	0.090	0.072
125	N5_25	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	3.139	5.592	3.953
126	N5_26	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.010	0.019	0.029
127	N5_27	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.005	0.013	0.015
128	N5_28	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	7.607	>10.24	6.899
129	N5_29	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.026	0.046	0.065
130	N5_30	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.018	0.023	0.061
131	N5_31	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.034	0.036	0.061
132	N5_32	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	5.918	8.960	6.037
133	N5_33	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	6.851	7.410	4.996
134	N5_34	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.018	0.022	0.056
135	N5_357	?	?	?	?	?	?	?	?	?	6.799	>10.24	7.111
136	N5_367	?	?	?	?	?	?	?	?	?	7.664	>10.24	7.233
137	N5_37	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.034	0.029	0.072
138	N5_38	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	6.952	7.935	6.417
139	N5_39	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	6.038	6.297	5.778
140	N5_40	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	3.378	4.699	2.404
141	N5_41	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	2.596	3.825	2.489
142	N5_42	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	2.565	4.474	2.026
143	N5_43	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	1.810	3.084	1.288
144	N5_44	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	7.370	>10.24	4.511

number	Isolate	Insertions in the promoter of the *cyp51* gene	Amino acid CYP51 substitutions								DMI fungicide EC_{50} average scores		
		Promoter	T18	V106	Y136	A313	H380	A381	Y463	A446	Difenoconazole	Epoxiconazole	Propiconazole
145	N5_45	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	6.778	8.602	3.462
146	N5_46	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	6.768	8.069	2.540
147	N5_47	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	4.868	3.809	4.303
148	N5_48	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.074	0.090	0.084
149	N5_49	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.090	0.110	0.096
150	N5_50	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.008	0.016	0.024
151	N5_51	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.015	0.016	0.045
152	N5_52	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.012	0.018	0.042
153	N5_53	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	2.270	5.514	1.574
154	N5_54	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.084	0.056	0.078
155	N5_55?	?	?	?	?	?	?	?	?	?	1.543	5.979	1.380
156	N5_56	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.015	0.015	0.043
157	N5_57	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.108	0.315	0.273
158	N5_58	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	1.250	1.673	1.672
159	N5_59	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.039	0.070	0.064
160	N5_60?	?	?	?	?	?	?	?	?	?	2.497	2.415	2.751
161	N5_61	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	1.277	2.034	1.657
162	N5_62	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	0.759	0.870	1.091
163	N5_63	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	3.711	4.724	1.836
164	N5_64	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.094	0.087	0.080
165	N5_65	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.074	0.069	0.094
166	N5_66	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.087	0.070	0.076
167	N5_67	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.013	0.015	0.034
168	N5_68	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	1.340	2.321	1.718
169	N5_69	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.018	0.021	0.047
170	N5_70	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	6.878	8.358	4.955
171	N5_71	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.053	0.090	0.083

number	Isolate	Insertions in the promoter of the *cyp51* gene	Amino acid CYP51 substitutions						DMI fungicide EC_{50} average scores		
		Promoter	T18	V106	Y136	A313	H380	A381	Y463	A446	Difenoconazole	Epoxiconazole	Propiconazole
172	N5_72	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	5.592	7.162	6.173
173	N5_73	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	4.497	4.222	4.500
174	N5_74	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.027	0.038	0.074
175	N5_75	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	5.290	4.509	5.688
176	N5_76	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	6.004	5.679	6.047
177	N5_77	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	5.330	5.347	5.224
178	N5_78	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.005	0.015	0.021
179	N5_81	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.048	0.054	0.063
180	N5_82	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.016	0.017	0.041
181	N5_83	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	2.634	6.064	3.011
182	N5_84	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	4.754	5.864	3.780
183	N5_85	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.080	0.094	0.088
184	N5_86	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	5.153	5.370	2.111
185	N5_87	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	6.803	8.057	6.775
186	N5_88	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.061	0.087	0.079
187	N5_89	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	2.449	4.478	2.317
188	N5_90	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.018	0.020	0.055
189	N5_91	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	3.130	4.263	3.355
190	N5_92	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.029	0.036	0.074
191	N5_93	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	1.491	4.167	3.211
192	N5_94	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.018	0.024	0.062
193	N5_95	AAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT ACGATGTTAAATCTCGTACGATAGCATAAATCTCGTACGATAGCATAAATCTCGT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	2.557	3.482	2.635

number	Isolate	Insertions in the promoter of the *cyp51* gene	Amino acid CYP51 substitutions								DMI fungicide EC_{50} average scores		
		Promoter	T18	V106	Y136	A313	H380	A381	Y463	A446	Difenoconazole	Epoxiconazole	Propiconazole
194	N5_96	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	6.681	7.430	5.895
195	N5_97	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.065	0.077	0.107
196	N5_98	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	7.336	9.459	7.229
197	N5_99?	?	?	?	?	?	?	?	?	?	5.599	6.688	2.938
198	N5_100	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	5.702	4.959	5.381
199	N5_101	AAATCTCGT ACGATAGCAT AAAATCTCGT ACGATAGCAT AAAATCTCGT ACGATGTTAA ATCTCGTACG ATAGCATAAA TCTCGTACGA TAGCACCTGC CCAT	T18I	V106D	WT	WT	H380N	A381G	Y463D	WT	2.803	3.863	3.281
200	N5_102	WT	T18I	V106D	WT	WT	WT	WT	WT	A446S	0.030	0.028	0.069

Table S3. Information of the 53 putative genes found in the overlapping genetic window of the *Pseudocercospora fijiensis* N2 and N5 mapping populations that contains the sensitivity region. The information of the genes was collected from the Joint Genome Institute, *Mycosphaerella fijiensis (Pseudocercospora fijiensis)* v2.0 portal. http://genome.jgi.doe.gov/Mycfi2/Mycfi2.home.html.

Transcript ID:	Location:	Gene ontology	Definition
177154	scaffold_7:1909687-1917514 (+)	Putative protein	No annotation yet
177158	scaffold_7:1938540-1939146 (-)	Putative protein	No annotation yet
31195	scaffold_7:1967582-1967875 (+)	Putative protein	No annotation yet
31837	scaffold_7:1920971-1921141(+)	Putative protein	No annotation yet
22691	scaffold_7:1949446-1950451 (-)	GO:0004553 hydrolase activity, hydrolyzing O-glycosyl compounds. Catalysis of the hydrolysis of any O-glycosyl bond.	No annotation yet
189819	scaffold_7:1929097-1930398 (-)	GO:0003824 catalytic activities. Catalysis of a biochemical reaction at physiological temperatures.	No annotation yet
30851	scaffold_7:1959643-1961193 (-)	Putative protein	No annotation yet
101452	scaffold_7:1931010-1931678 (-)	Putative protein	No annotation yet
101452	scaffold_7:1931010-1931678 (-)	Putative protein	No annotation yet
211990	scaffold_7:1943313-1944619 (-)	GO:0000172 ribonuclease MRP complex. A ribonucleoprotein complex that contains an RNA molecule of the snoRNA family, and cleaves the rRNA precursor as part of rRNA transcript processing. It also has other roles: In S. cerevisiae it is involved in cell cycle-regulated degradation of daughter cell-specific mRNAs, while in mammalian cells it also enters the mitochondria and processes RNAs to create RNA primers for DNA replication.	No annotation yet
7504	scaffold_7:1939638-1940268 (+)	Putative protein	No annotation yet
77954	scaffold_7:1945205-1946623 (-)	GO:0004553 hydrolase activity, hydrolyzing O-glycosyl compounds. Catalysis of the hydrolysis of any O-glycosyl bond.	No annotation yet
31273	scaffold_7:1947348-1948031 (-)	GO:0004733 pyridoxamine-phosphate oxidase activity. Catalysis of the reaction: pyridoxamine 5'-phosphate + H2O + O2 = pyridoxal 5'-phosphate + NH3 + hydrogen peroxide.	No annotation yet
198484	scaffold_7:1951727-1952825 (-)	Putative protein	No annotation yet
77958	scaffold_7:1953723-1954161 (-)	Putative protein	No annotation yet
77959	scaffold_7:1954646-1955608 (+)	Putative protein	No annotation yet
198487	scaffold_7:1955999-1956492 (+)	Putative protein	No annotation yet
204493	scaffold_7:1957874-1958706 (+)	Putative protein	No annotation yet
77962	scaffold_7:2016030-2016929 (-)	Putative protein	No annotation yet

Transcript ID:	Location:	Gene ontology	Definition
177166	scaffold_7:2021508-2023032 (+)	Putative protein	No annotation yet
177167	scaffold_7:2026479-2029163 (+)	Putative protein	No annotation yet
177169	scaffold_7:2032413-2034139 (+)	Putative protein	No annotation yet
77963	scaffold_7:2035236-2035724 (+)	Putative protein	No annotation yet
77964	scaffold_7:2036475-2038341 (-)	Putative protein	No annotation yet
208509	scaffold_7:2038381-2039739 (+)	Putative protein	No annotation yet
155993	scaffold_7:2039032-2039971 (-)	GO:0003723 RNA binding. Interacting selectively and non-covalently with an RNA molecule or a portion thereof.	No annotation yet
49844	scaffold_7:2040187-2041265 (+)	GO:0004298 threonine-type endopeptidase activity. Catalysis of the hydrolysis of internal peptide bonds in a polypeptide chain by a mechanism in which the hydroxyl group of a threonine residue at the active centre acts as a nucleophile.	No annotation yet
96804	scaffold_6:4257330-4259091 (-)	GO:0003677 DNA binding. Any molecular function by which a gene product interacts selectively with DNA (deoxyribonucleic acid). GO:0003700 sequence-specific DNA binding transcription factor activity. Interacting selectively and non-covalently with a specific DNA sequence in order to modulate transcription. The transcription factor may or may not also interact selectively with a protein or macromolecular complex. GO:0006355 regulation of transcription, DNA-dependent. Any process that modulates the frequency, rate or extent of cellular DNA-dependent transcription.	No annotation yet
166575	scaffold_7:2042679-2044011 (+)	GO:0005515 protein binding. Interacting selectively and non-covalently with any protein or protein complex (a complex of two or more proteins that may include other non-protein molecules).	No annotation yet
77971	scaffold_7:2049509-2051776 (-)	Putative protein	No annotation yet
31107	scaffold_7:2054261-2055433 (+)	GO:0003824 catalytic activity. Catalysis of a biochemical reaction at physiological temperatures. In biologically catalysed reactions, the reactants are known as substrates, and the catalysts are naturally occurring macromolecular substances known as enzymes. Enzymes possess specific binding sites for substrates, and are usually composed wholly or largely of protein, but RNA that has catalytic activity (ribozyme) is often also regarded as enzymatic.	No annotation yet
204500	scaffold_7:2056817-2057682 (+)	Putative protein	No annotation yet
156006	scaffold_7:2058401-2059893 (-)	GO:0000166 nucleotide binding. Interacting selectively and non-covalently with a nucleotide, any compound consisting of a nucleoside that is esterified with (ortho)phosphate or an oligophosphate at any hydroxyl group on the ribose or deoxyribose moiety.	No annotation yet

Transcript ID:	Location:	Gene ontology	Definition
49849	scaffold_7:2060248-2062273 (+)	GO:0000015 phosphopyruvate hydratase complex. A multimeric enzyme complex, usually a dimer or an octamer, that catalyses the conversion of 2-phospho-D-glycerate to phosphoenolpyruvate and water.	No annotation yet
208514	scaffold_7:2044371-2048954 (-)	GO:0008060 ARF GTPase activator activity. Increases the rate of GTP hydrolysis by the GTPase ARF.	No annotation yet
77972	scaffold_7:2052410-2053032 (-)	Putative protein	No annotation yet
31190	scaffold_7:2055461-2056561 (-)	GO:0003824 catalytic activity. Catalysis of a biochemical reaction at physiological temperatures. In biologically catalysed reactions, the reactants are known as substrates, and the catalysts are naturally occurring macromolecular substances known as enzymes. Enzymes possess specific binding sites for substrates, and are usually composed wholly or largely of protein, but RNA that has catalytic activity (ribozyme) is often also regarded as enzymatic.	No annotation yet
211997	scaffold_7:2062352-2063340 (-)	Putative protein	No annotation yet
198503	scaffold_7:2063728-2064756 (+)	Putative protein	No annotation yet
31199	scaffold_7:2082954-2084195 (-)	GO:0006508 proteolysis. The chemical reactions and pathways resulting in the breakdown of a protein by the destruction of the native, active configuration, with the hydrolysis of peptide bonds.	No annotation yet
156013	scaffold_7:2084798-2087077 (+)	GO:0004672 protein kinase activity. Catalysis of the phosphorylation of an amino acid residue in a protein, usually according to the reaction: a protein + ATP = a phosphoprotein + ADP.	No annotation yet
77984	scaffold_7:2088199-2089353 (-)	Putative protein	No annotation yet
211999	scaffold_7:2096114-2096935 (-)	Putative protein	No annotation yet
63438	scaffold_7:2122388-2122960 (+)	Putative protein	No annotation yet
104048	scaffold_7:2090006-2095772 (-)	GO:0005488 binding. The selective, non-covalent, often stoichiometric, interaction of a molecule with one or more specific sites on another molecule.	No annotation yet
156019	scaffold_7:2115877-2116567 (+)	Putative protein	No annotation yet
212000	scaffold_7:2117112-2119076 (+)	Putative protein	No annotation yet
204508	scaffold_7:2117112-2119587 (-)	Putative protein	No annotation yet
30715	scaffold_7:2119919-2121685 (-)	GO:0004497 monooxygenase activity. Catalysis of the incorporation of one atom from molecular oxygen into a compound and the reduction of the other atom of oxygen to water. GO:0005506 iron ion binding. Interacting selectively and non-covalently with iron (Fe) ions. GO:0006118 electron transport. OBSOLETE. The transport of electrons from an electron donor to an electron acceptor. GO:0020037 heme binding. Interacting selectively and non-covalently with heme, any compound of iron complexed in a porphyrin (tetrapyrrole) ring.	Lanosterol 14α-demethylase, catalyses the C-14 demethylation of lanosterol in the ergosterol biosynthesis pathway. Target for azole fungicides (Rafael Arango, 2008-11-10). Name: CYP51 (Rafael Arango, 2008-11-10)

Transcript ID:	Location:	Gene ontology	Definition
63438	scaffold_7:2122388-2122960 (+)	Putative protein	No annotation yet
212002	scaffold_7:2124095-2125034 (+)	Putative protein	No annotation yet
86816	scaffold_7:2125667-2128069 (+)	GO:0005215 transporter activity. Enables the directed movement of substances (such as macromolecules, small molecules, ions) into, out of or within a cell, or between cells. GO:0006810 transport. The directed movement of substances (such as macromolecules, small molecules, ions) into, out of or within a cell, or between cells, or within a multicellular organism by means of some agent such as a transporter or pore. GO:0016020 membrane. Double layer of lipid molecules that encloses all cells, and, in eukaryotes, many organelles; may be a single or double lipid bilayer; also includes associated proteins.	No annotation yet
77993	scaffold_7:2128129-2130717 (-)	Putative protein	No annotation yet

Chapter 6

General discussion

General discussion

Fungicides are currently key tools for disease control. Among the fungicides, triazoles (azoles) belonging to the group of demethylation inhibitors (DMIs) are ubiquitous compounds for the control of human and plant diseases (Chowdhary *et al.* 2013; Cools *et al.* 2013; Ploetz *et al.* 2015; Verweij *et al.* 2013). Black Sigatoka control in banana, caused by *Pseudocercospora fijiensis*, relies on intensive application of triazoles in fungicide mixtures, next to cultural measures such as deleafing (Cañas *et al.* 2009; Marín *et al.* 2003; Pérez 2006). Although the disease is still manageable, the appearance and spread of resistant strains is alarming (Cañas *et al.* 2009; Churchill 2011b; Ploetz *et al.* 2015). The single target DMIs fungicides, targeting the 14α-demethylase enzyme, together with the sexual reproduction of *P. fijiensis* have greatly contributed to this phenomenon. The increasing problem of reduced efficacy of DMI fungicides to *P. fijiensis* urges for understanding of the underlying developmental mechanisms to ensure successful future control strategies based on similar and new chemistries. While resistance monitoring measures are generally applied in banana farms worldwide, the methods are outdated and key genetic information is hardly available, which translates into uncertainty and routine fungicide application rather than into decision support mechanisms. The aim of this thesis was, therefore, to elucidate the molecular mechanisms of reduced efficacy to DMI fungicides in *P. fijiensis*.

DMI fungicides selective pressure

For years, the DMI baseline sensitivity in most banana producing countries is continuously rising. Marín et al., (2003) reported an average EC_{50} for propiconazole of 0.15 $mg.L^{-1}$ with a maximum value of 0.5 $mg.L^{-1}$ in Costa Rican populations. In 2009, our study

revealed an increase in the propiconazole EC_{50} values to an average of 1.10 $mg.L^{-1}$ with a maximum value of 1.53 $mg.L^{-1}$ for four resistant Costa Rican isolates (Chapter 3, (Díaz-Trujillo *et al.* 2016a)). A subsequent analysis of 107 *P. fijiensis* isolates from 2014 showed again an increase in resistance with an EC_{50} average of 5.8 $mg.L^{-1}$ and a maximum value of 18.4 $mg.L^{-1}$ for propiconazole (Chapter 2, (Chong *et al.* 2016b)). These data revealed an ~40-fold increase of the EC_{50} over a decade, which was gradual and hence, predictable. Costa Rica has a long history of fungicide use in black Sigatoka management (Marín *et al.* 2003) and the DMI baseline sensitivity shift correlates with the increasing amounts of fungicides being used. The number of fungicide applications raised from 30 in the 90's up to 50 treatments by 2007 and are still rising (Lapeyre *et al.* 2010a). The results from the Costa Rican isolates are representative for the selective pressure role exerted by DMIs fungicides on the pathogen population. In this thesis we elaborated on the DMIs sensitivity baseline for *P. fijiensis* isolates representing populations from various countries. Unfortunately, documentation on the development of DMI sensitivity, in combination with the number of application is frequently lacking. Nonetheless, populations derived from countries with a (long) history of DMI applications are generally resistant. In contrast, *P. fijiensis* populations from areas without fungicide applications are generally sensitive. Exemplary are indigenous areas such as Bohol, Philippines, or the San Pablo area of Costa Rica as well as the sensitive populations from the most recently colonized areas, Martinique and Guadalupe (Guzmán *et al.* 2013; Ioos *et al.* 2011). This latter case raises questions about their origin. As stated in Chapter 2, in order for all isolates in these populations to be sensitive they should fit one of the two following hypotheses: (1) the islands were colonized by wild type (wt) *P. fijiensis* populations that had not undergone DMI selection pressure or (2) the islands were colonized by *P. fijiensis* populations that had undergone DMI selection pressure, but they reverted to wild type populations due to the lack of DMI selective pressure. The latter case supports the fitness costs

theory where DMI resistance is accompanied with a fitness penalty, which is rapidly lost in the absence of selective pressure. This phenomena was observed for *Magnaporthe oryzae* and *Cercospora beticola* but remained unnoticed for many others fungi (Hollomon 2015). Moreover, the isolates from Martinique and Guadalupe are closely related to the Latin American population, which might support hypothesis 2. However, in both island the selective pressure exist since DMI are used to control yellow Sigatoka. Besides, this population are also sensitive to other fungicides, namely strobilurins (QoI) and benzimidazoles (MBC) (data not shown) thus, the favoured hypothesis is that these islands were colonized by wt *P. fijiensis* isolates. We, therefore, consider the fitness hypothesis unlikely for *P. fijiensis*, particularly since we have identified wt strains in other non-sprayed areas such as San Carlos (Arango *et al.* 2016) in Costa Rica and Bohol in the Philippines as well as in Cameroon, Colombia, and Ecuador (Chapter 2). Nevertheless, it is worth mentioning that our research was limited to just a few isolates from these populations, hence analyses on more isolates and further genetic studies are required to conclusively elucidate this matter.

The role of selection exerted by DMI fungicides on *P. fijiensis* population is highlighted in Chapter 2 and 3 of this thesis in which we studied the genetic and phenotypic response of a global panel of *P. fijiensis* isolates to DMIs. The global panel strain from countries with a wide diversity in the intensity of DMI fungicide applications. This allowed us to compare the effects of fungicide application on DMI efficacy, i.e. the distribution of resistant and sensitive *P. fijiensis* strains and to analyse the underlying genetic background. Consequently, most resistant strains were collected from countries where banana production is important, which suffer from black Sigatoka disease and hence are exposed to a high fungicide application frequency. This information enables us now to predict, based on the number of fungicide application cycles and the country origin, the level of DMI sensitivity.

We show that DMI resistance in *P. fijiensis* correlates with specific changes in the *Pfcyp51* gene.

CYP51 structure and DMI resistance

The accumulation of modifications in the CYP51 protein tends to confer reduced efficacy of DMI fungicides in several organisms (Cools *et al.* 2013). A high degree of polymorphisms in CYP51 was previously reported for *Tapesia acuformis* and *T. yallundae* (Albertini *et al.* 2003). We also identified a high degree of polymorphisms in *P. fijiensis* with 60 different *Pfcyp51* genotypes resulting in 28 different amino acid (aa) substitutions in the resulting protein. Among these, aa substitutions resulting in A19E, I70M, D71E, V260L, I264T, H380N, R418G, D460E, D460V, Y461N, Y461S, ΔY461 and G462D were hitherto unpublished in *P. fijiensis.* However, not all of these substitutions correlate with DMI resistance. Surprisingly, we identified sensitive isolates carrying three aa CYP51 modifications, which apparently might represent natural random mutations, although some could be compensatory substitutions. Substitutions T18I and A446S had an additive (EC_{50}) effect in tolerant and resistant isolates. Additive compensatory substitutions were nicely illustrated by aa changes in *Zymoseptoria tritici* - the Septoria tritici blotch pathogen of wheat - since the main substitution for fungicide resistance was enzymatically lethal as corroborated by complementation experiments in *Saccharomyces cerevisiae* (Becher & Wirsel 2012). Future studies in fungi showing reduced efficacy to DMIs should elaborate on the role of substitutions outside the catalytic core of the CYP51 protein.

The effect of DMI applications on genetic changes of *P. fijiensis* is exemplified by key mutations in the *Pfcyp51* gene. Especially CYP51 substitution A313G, and to a lesser extend Y136F, H380N, Y463D and D460V correlate with DMI resistance (Chapter 2). Equivalent substitutions in CYP51 of other fungi confirm their vital role in azole resistance

(Albertini *et al.* 2003; Cools *et al.* 2013; Délye *et al.* 1997; Lupetti *et al.* 2002). The importance of substitutions located in or near the core of CYP51 was highlighted by CYP51 modelling and fungicide docking experiments (Chapter 2). According to Mullins et al. (2011) DMI resistance due to modulation of the CYP51 enzyme occurs in the following order: (1) obstruction or loss of interaction due to residue substitution; (2) constriction of the binding cavity to block the access of azoles; and (3) enlargement of the binding cavity to prevent interactions between key residues and the active ingredient (Mullins *et al.* 2011). Interestingly, CYP51 modelling and propiconazole docking experiments confirmed all these options. Substitutions at positions 136, 313, 380, 381 affect the core of the protein in the substrate recognition site (SRS) and fulfil Mullins first statement. Modulations at positions 460 to 463, not located in the SRS, compromise the three-dimensional structure of the protein resulting in an affinity change due to significant distance and angle changes around position 524 to 526 (SRS6) (Chapter 2). Similar observations were made for the deletion of Y461 affecting the active site (Chapter 2). Position 125, at the entrance of the channel to the active site of the protein, was modified in all resistant strains, resulting in constriction of the binding cavity or enlargement of the binding cavity. Either affects the protein affinity for the active ingredient of the fungicide (Mullins *et al.* 2011). This knowledge can now be applied in protein modelling, anticipating on specific substitutions, and the effect on binding of azole fungicides and hence, act as a prediction tool in order to develop azole based compounds *de novo*.

Interestingly, the *P. fijiensis* reference strain CIRAD86 that was used to generate the first linkage map (Manzo-Sanchez *et al.* 2008) that was also used and improved in a recent whole genome sequencing project (Kema 2009), is the only strain among 268 isolates that encodes a valine instead of aspartic acid at position 106 in CYP51 (Chapter 2). Since South East Asia is the proposed centre of origin (Carlier *et al.* 1996; Halkett *et al.* 2010; Hayden *et al.* 2003; Rivas *et al.* 2004b) and CIRAD86 originates from Cameroon, we concluded that

substitution D106V is a rare event that exclusively occurred in CIRAD86 rather than the reverse substitution in all other strains. The proposed additive role of D106V, as stated in Chapter 2, is most likely a statistical artefact based on another (other) mutation(s) in the reference strain. Due to the fact that all resistant strains possess this variant, the statistical variance of V106D + A313G is, with 11.8, slightly higher than the threshold of 10. However, this is likely a statistical artefact as the interaction was preferred by the algorithm, probably by the presence of the resistant substitution A313G rather than V106D. This highlights two important issues: first, one should always consider biology for final decisions to avoid miss-interpretation based on probability; secondly, although the CIRAD86 genome has been a priceless tool for our studies, there is an urgent need for sequencing many more *P. fijiensis* isolates, preferably using strains from the centre of origin, to better understand overall genetic diversity.

Discovery of *Pfcyp51* promoter insertions and their role in reduced efficacy of DMIs

Whether aa substitutions are the main mechanism for reduced DMI efficacy is addressed in Chapter 3. For the first time we observed overexpression of the target *Pfcyp51* gene, apparently through (repeated) insertions of a *Pfcyp51* promoter localized sequence. The inserted sequences are composed of a particular repeat element (Chapter 2 and 3), which are widely shared among tolerant and resistant strains, whereas it was absent from all sensitive strains. The presence of these insertions and their number, positively correlate with DMIs fungicide resistance (Chapter 2). Since their discovery in Costa Rica populations (Chapter 3), other populations were identified with similar insertions (Chapter 2). In a previous study, the role of this mechanism was thought to be negligible since overexpression of the *Pfcyp51* gene in propiconazole resistant isolates from Colombia was not observed (Cañas *et al.* 2009). There

are however, many possible reasons why overexpression was not (yet) observed: firstly, the propiconazole resistance level was still much lower than we observed for resistant strains (Chapter 2 and 3); and secondly, the Colombian set of isolates analysed by Cañas-Gutierrez (2009) was smaller. It is plausible that the frequency of strains, sampled in 2008, with insertions, was negligible or even not-existing (Cañas-Gutierrez 2009). In contrast, among the 2012 Colombian derived *P. fijiensis* strains, 24 out of 34 resistant strains contained a promoter insertion (70%). This rapid increase, between 2008 to 2012, in both EC_{50} values and promoter insertion frequency correlated with the constant selection pressure of DMI fungicide applications with an average of 6.8 cycles per year (6.8 from a total of 30 fungicide applications) from 2008 to 2012 (Vicente Rey, Augura, personal communication). These data could be used to extrapolate the critical DMI selection pressure for the appearance of a particular resistant mechanism within a given population. Such a tool would be very useful for the management and improvement of diseases control strategies.

Inserts as observed in *Pfcyp51* promoters are commonly observed in other fungal species (Cools *et al.* 2012; Hamamoto *et al.* 2000; Ma *et al.* 2006; Mellado *et al.* 2007; Schnabel & Jones 2000; Snelders *et al.* 2012; Villani *et al.* 2016). Nevertheless, they differentiate greatly between species based on their size, sequence, position and nature. Clearly, they are the result of independent events, raising important questions about their origin. Some insertions are considered to be remains from transposable element activity. These can contain powerful promoter sequences whose footprints could be the observed insertions (Cools et al., 2013). The repeats identified in *P. fijiensis* can be categorized as mini-satellite like structures (>14 bp), as found by Espley et al. (2009) in the promoter of MYB10 overexpressed in red flesh apples (Espley *et al.* 2009). The expansion mechanism of mini-satellites is suggested to result from recombination (Espley *et al.* 2009). Nonetheless, mini-satellite like structures have been related to miniature inverted-repeat transposable elements

(Espley *et al.* 2009; Lu *et al.* 2008). Since the *Pfcyp51* gene is 8.7 kb away from the nearest recombination point (Chapter 4), it is unlikely that recombination is the key player in the *Pfcyp51* repeat element expansion and also, there is no clear evidence for miniature inverted-repeat transposable elements, and hence, the origin of these repeats remains unknown.

The *P. fijiensis* repeat element's central core is a palindromic motif. These motifs are frequently annotated as cis-elements, an important group of regulators in eukaryotes (Knox & Keller 2015). It is well known that many transcription factors (TF) bind palindromic sequences with high affinity (Narlikar & Hartemink 2006; Qian *et al.* 2006). Interestingly, we observed that these elements negatively regulate fungicide efficacy: an increasing number of repeat elements, particularly with the number of palindromic sequences, reduced the efficacy (Chapter 2 and 3). Finally, the promoter swapping transformation experiments described in Chapter 3 proved that the causality of these insertions: insertion in the *Pfcyp51* promoter both increased the gene's expression as well as azole resistance. This is consistent with observations in *Venturia inaequalis* where overexpression of the *cyp51* confers differential resistance to difenoconazole (Villani *et al.* 2016). However, the increase of DMI resistance in the *P. fijiensis* mutants was not as high as in the resistant wt strain (Chapter 3) since that strain also possessed aa modulations in the target *Pfcyp51* gene. So, overexpression synergizes the effect of accompanying effective target site mutations. All these observations are consistent with results from *Z. tritici*, where promoter insertions were suggested to occur after target site mutations and also prevented further accumulation of such mutations as this would eventually compromise the enzymatic activity and stability of the protein (Leroux and Walker, 2011). Finally, in Chapter 2 the statistical analyses on the role of promoter insertions in reduced DMI efficacy revealed that they are not the main explanatory component, but important add-ons to key target site mutations.

One of the main upcoming questions for *P. fijiensis* is what molecular machinery drives this increase of repeat elements? Whole genome sequence methodology can shed light to their origin, but except for the palindromic sequence, no general lead was found. Therefore, we hypothesize that these palindromes are essential for further repeat amplification. One possible mechanism to study the role of the element and its palindromic core is DNA-protein hybridization. In particular, yeast one hybrid has been a useful DNA-protein hybridization system to find transcription factors (Ota *et al.* 2014). We could try using the *Pfcyp51* promoter as a capture probe in a genome-wide mapping of promoter-anchored interactions through HiCap methodologies for the identification of regulatory interactors as this is based on modified chromosome conformation capture followed by a sequence-capture of promoter containing fragments, resulting in a high-resolution map of promoter-anchored interactions (Sahlen *et al.* 2015). Finally, to elucidate more basic elements in fungal promoters, cap analysis of gene expression (CAGE) technology can be used to detect transcriptional start site(s) (TSS) and the expression levels by utilizing 5' cDNA tags and PCR (Kurosawa *et al.* 2011).

Classical genetic analysis to unequivocally identify and map genomic regions involved in DMIs resistance

The aim of Chapter 4 was to elucidate the genetic nature of reduced DMI efficacy by genetic mapping using segregating *P. fijiensis* populations from crosses between isolates with major differences in DMI sensitivity. We, therefore, generated, phenotyped and genotyped two mapping population and constructed two linkage maps using Diversity Array Technology (DArT) markers. As discussed earlier in Chapter 4, a gradual shift from sensitivity to resistance is usually based on the interaction of many genes, often referred to as quantitative or polygenic resistance (Dyer *et al.* 2000). The DMI resistance mechanism was characterized as polygenic

for *Candida albicans, A. fumigatus* and *Z. tritici* (Cools *et al.* 2013; Cowen 2008). Also, reduced DMI sensitivity for *P. fijiensis* in the field has been gradual in nature (Cañas *et al.* 2009; Marín *et al.* 2003). Nevertheless, the evidence presented in this thesis points at *Pfcyp51* as the single major gene involved and hence, the quantitative expression of the phenomenon seems to be largely due to the various modulations of the CYP51 protein and the binding of various active ingredients.

The first evidence of a monogenic cause in *P. fijiensis* was the correlation of the *Pfcyp51* changes with reduced DMIs efficacy as described in Chapter 2. The second evidence is the clear distinct 1:1 segregation for DMI sensitivity and analogous *Pfcyp51* modifications in the *P. fijiensis* mapping populations described in Chapter 4. Thirdly, the genetic maps revealed one genetic region harbouring *Pfcyp51* as the single most important candidate gene. Nonetheless, subtle variations were observed between individuals with the same *Pfcyp51* genotype configuration. We, therefore, cannot exclude modifying factors for DMI sensitivity, including physiological factors such as colony age, growth ratios and other stress factors.

Modernizing monitoring

Hitherto, DMI efficacy is monitored by germ tube lengths measurement and germination ratios of *P. fijiensis* ascospores. Though technically simple, it does not provide any insight in the underlying mechanisms and hence, DNA based methodologies are preferred to further precise and modernize monitoring strategies. Rapid molecular monitoring tools such as PCR-based technologies reduce the required timeframe for an adequate response to any disease. For DMI efficacy monitoring we could develop a quick molecular test focusing on the presence of important substitutions in PfCYP51, e.g. position 313, 136 and 463. We have shown that a simple PCR, which is based on the variable number of the insertions, indicates the presence of *Pfcyp51*promoter repeats, suggesting the potentially reducing sensitivity levels

in natural *P. fijiensis* populations (Chapter 3) for instance in Costa Rican populations (Díaz-Trujillo *et al.* 2016a). Ideally, such test should be run directly on leaf tissue, which would further the implementation of technology revealing the genomic basis of DMI resistance in *P. fijiensis* strains. The generation of the two genetic maps based on DMI sensitivity (Chapter 4) also contributed significantly to the identification of molecular markers and candidates genes involved in DMI sensitivity for further studies (Chong *et al.* 2016c). These should include quantification of DNA/RNA species enabling the ratio of resistant vs. wt genotype(s) in natural population (Singh & Mustapha 2013).

Concluding remarks

In conclusion, the proven monogenic basis of DMI sensitivity in *P. fijiensis* has an apparent quantitative phenotypic expression, which in many systems is considered to result from a polygenically controlled mechanism (Cools & Fraaije 2013; Cools *et al.* 2013; Dyer *et al.* 2000). How can we reconcile such a seemingly contrasting observation? Interestingly as noted in Chapter 2, each of the *Pfcyp51* mutations contributes to resistance, but does not confer full DMI resistance as it seems to depend on the balance between catalytic activity of the CYP51 protein and the active ingredient of the fungicide. Another phenomenon is that a particular substitution affects individual, or subsets of DMIs compounds, but is insufficient for cross-resistance to the whole class of fungicides (Cools et al., 2013), which was nicely illustrated in *Venturia inaequalis* with differential resistance to difenoconazole and myclobutanil (Villani *et al.* 2016). In theory, each (surviving) mutation in the CYP51 protein will be based on the interaction with the DMIs applied in the field. However, this is insufficient for full resistance against the array of fungicides.

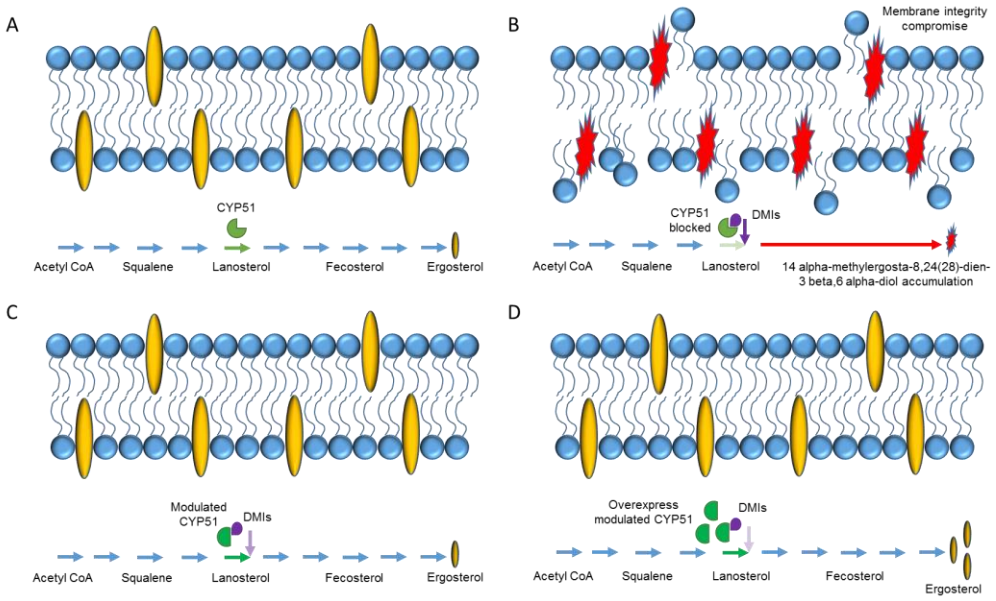

Figure 1. Schematic representation of CYP51 interaction with DMI fungicides in *Pseudocercospora fijiensis* based on Cowen et al (2008) *cyp51* illustration. A) Normal membrane integrity and ergosterol pathway. B) Scheme of the effect of DMI fungicides on the membrane integrity and the ergosterol pathway. Accumulation of 14 α-methylergosta-8,24(28)-dien-3 β, 6 α-diol will stop development and cause cell death (Lupetti *et al.* 2002; Shapiro *et al.* 2011). C) Modulated CYP51 reduces the affinity for the interaction with DMI fungicides and thereby their efficacy, hence increasing amounts of fungicide are needed for disease control. D) Increased expression of CYP51 overcomes increasing amounts of fungicides. This increment of enzymatic active units causes a synergistic effect with the reduced affinity towards the fungicide, resulting in a further amplification of resistance. In comparison with scenario (C) an increases of fungicide doses from ten times or more will be required for effective disease control (D).

The discovered *Pfcyp51* overexpression mechanism is the latest novelty in an important research area. Overexpression is unavoidable to maintain catalytic activity under the mutational pressure at and around the catalytic site (Figure 1). Finally, we hypothesize that in the near future additional mechanisms will appear, such as the increased exclusion of fungicides from the intracellular compartments as observed before in other DMI stressed fungi (Cowen 2008). Potentially, the occurrence of this mechanism in *P. fijiensis* might provoke a non-gradual increase in DMI resistance, which cannot be counteracted by increased fungicide

applications and hitherto practiced (Chapter 2). This stresses the need for novel mode of action fungicides (moa's) or control strategies to manage black Sigatoka disease in the future. The knowledge of the current distribution, evolution and impact of the resistance in the field is therefore an invaluable data source for the future control of this important banana disease.

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Summary

Pseudocercospora fijiensis is the causal agent of black Sigatoka which is the most serious leaf defoliation disease on *Musa* spp. (bananas and plantains). Many plantain and banana species are susceptible to black Sigatoka including the exporting Cavendish cultivars. Leaf defoliation results in significant yield loses and premature ripening of banana fruit, which is a serious problem for the banana exporting industry. The main control measure of black Sigatoka involves frequent fungicide application with a very high environmental and economic burden. Among these fungicides, the azole chemical family is one of the most frequently used fungicides for the control of the disease. Azole fungicides belong to the sterol demethylation-inhibitors (DMIs) that target the lanosterol 14α-demethylase enzyme (CYP51). One of the major problems in black Sigatoka control has been the excessive and unplanned use of the DMI fungicide applications in many banana farms worldwide. This uncurbed use of the fungicide resulted in DMI resistance in pathogen population. Over time, resistance levels have increased to such an extent that the number of fungicide application cycles is now near maximum level. The reduction of sensitivity in *P. fijiensis* to currently used DMIs has been gradual in nature, suggesting a polygenic control (Cañas *et al.* 2009). Nevertheless, genetic evidence described in this thesis suggests that *Pfcyp51* is the single major factor responsible for the sensitivity loss in the field. Our study is the first global analysis of DMI fungicide resistance in *P. fijiensis*, provides a lead to understand DMI sensitivity reduction, enables the development of better black Sigatoka management strategies, but also calls for more sustainable solutions of this unparalleled banana threat.

Chapter 1 describes the importance of the banana fruit as commodity and staple food worldwide and the impact of black Sigatoka on its cultivation. It introduces the subject of the thesis, the problem of the resistance to DMIs in the control of black Sigatoka and describes lifestyle features of the causal agent *P. fijiensis*, the history of fungicide control of the disease,

the impact that DMI fungicides exerted in the population of this species and concludes with the set-up of the thesis.

Chapter 2 provides an historical treatise of black Sigatoka management – primarily in Costa Rica – including the strategies that were developed and applied. It concludes with a critical evaluation of the current practice and the required changes.

Chapter 3 describes an extensive worldwide phenotypic and genotypic survey of *P. fijiensis* resistance to DMI fungicides. The sensitivity of a set of 592 field isolates collected from various banana production zones in Colombia, Costa Rica, the Dominican Republic, Ecuador, the Philippines, Guadalupe, Martinique and Cameroon was tested. The sequence analyses of the 14α-demethylase enzyme CYP51 encoding the *Pfcyp51* gene in 266 isolates showed a wide suite of modulations. Insertions of a 19 base pairs (bp) element found in the promoter region of the *Pfcyp51* gene were described and the correlation between these changes in the *Pfcyp51* gene and promoter and the increase in azole resistance was established. In addition, the contribution of the main CYP51 amino acid substitutions through the elucidation of seven in silico protein models was evaluated.

Chapter 4 describes the *de-novo* identification of a 19 bp element found in the promoter region of the *Pfcyp51* gene. Evidence strongly suggested that insertion of this element in the promoter - up to 6 copies - of resistant strains causes over expression of the *Pfcyp51* gene in comparison to strains that contain one element. PCR based assays were used to analyse the presence of the repeat element in four *P. fijiensis* populations of Costa Rica and some isolates from Ecuador, Africa and South East Asia. Promoter swap transformation experiments were used to analyse the role of the repeat element in the expression of the *Pfcyp51* gene. This identified the repeat element as a novel component that, together with mutations in the *Pfcyp51* open reading frame, are responsible for higher levels of resistance against azole fungicides.

Chapter 5 describes the generation of two F_1 *P. fijiensis* progenies for the construction of two genetic maps that identifies the region encoding for DMI fungicide resistance using DArTseq technology. Full agreement was found between the genetic markers in either population, underlining the robustness of the approach. This genetic tool was essential to identify the genetic region that determines the resistant to DMI fungicides in the species and strongly supports the hypothesis that the *Pfcyp51* gene is the single major determinant of

resistance towards DMI fungicides in *P. fijiensis*. The mapped region comprises 250,660 bp and contains 53 putative genes, including the *Pfcyp51* gene, which is the most plausible candidate as the driving molecular force for the resistance to DMI fungicides based on our and others' findings.

Chapter 6 discusses the experimental outcomes obtained in the thesis and describes them in a broader framework. It highlights the compelling evidence that modulation of the promoter and the coding gene sequence of *Pfcyp51* correlate with the observed azole sensitivity. Finally, the impact and implications of these findings are discussed for future disease control strategies.

Resumen

Pseudocercospora fijiensis es el agente causal de la Sigatoka negra, la enfermedad foliar más grave de *Musa* spp. (bananos y plátanos). Muchas especies de plátano y banano son susceptibles a la Sigatoka negra incluyendo los cultivares de exportación Cavendish. La defoliación que causa la enfermedad resulta en una reducción significativa de la producción y la maduración prematura de la fruta, que es un serio problema para la industria exportadora de banano. La principal medida de control de la enfermedad implica la aplicación frecuente de fungicidas con un impacto ambiental y económico muy alto. Entre los fungicidas usados para el control de la enfermedad, los triazoles son uno de los fungicidas más utilizados. Los azoles pertenecen al grupo de compuestos inhibidores de la des-metilación del esterol (DMIs). Estos fungicidas pertenecientes al grupo DMI que actúan directamente en la inhibición de la enzima lanosterol 14α-desmetilasa (CYP51). Uno de los principales problemas en el control de la Sigatoka negra ha sido el uso excesivo y no planificado de las aplicaciones de fungicidas DMI en muchas fincas de banano alrededor del mundo. Este uso desordenado de los fungicidas ha dado lugar a la aparición de resistencia a los DMI en las poblaciones del patógeno. Con el tiempo, los niveles de resistencia han aumentado a tal medida que el número de ciclos de aplicación de fungicidas están cerca del nivel máximo. La pérdida de sensibilidad de *P. fijiensis* a los DMI que se utilizan actualmente ha sido gradual, esto aparentemente sugeriría que la resistencia a los DMI es de naturaleza poligénica. Sin embargo, la evidencia genética encontrada en esta tesis sugiere que el gen *cyp51* es el principal y único responsable de la pérdida de sensibilidad en el campo. Este estudio es el primer análisis global de la resistencia a los fungicidas DMI en *P. fijiensis* y; ofrece pistas para entender la reducción de la sensibilidad a los DMI. La información obtenida en este trabajo nos permitirá el desarrollo de mejores estrategias de manejo de la Sigatoka negra, pero al mismo tiempo nos muestra la necesidad de la búsqueda de soluciones más sostenibles para lidiar con esta amenaza sin precedentes al cultivo del banano.

El **capítulo 1** describe la importancia de la fruta del banano como bien de exportación y como alimento básico a nivel mundial, mostrándonos el impacto que la Sigatoka negra ejerce en su cultivo. Nos introduce el tema de la tesis, el problema de la resistencia a DMIs en el control de la Sigatoka negra. Describe las características del estilo de vida del agente causal *P. fijiensis*, la historia del control de la enfermedad y el impacto que ejercen los fungicidas DMI en la población de la especie y concluye mostrando la estructura de la tesis.

El **capítulo 2** provee una disertación histórica del manejo de la Sigatoka negra – principalmente en Costa Rica – incluyendo las estrategias que han sido desarrolladas y aplicadas a través del tiempo. Concluye con una evaluación critica del presente manejo de la enfermedad y de los cambios que se necesitan para futuro.

El **capítulo 3** Describe un análisis fenotípico y genotípico mundial de la resistencia de *P. fijiensis* a los fungicidas DMIs. En el capítulo 2 se examinó la sensibilidad de un conjunto de 592 aislados del campo recogidos de diferentes zonas de producción bananera en Colombia, Costa Rica, República Dominicana, Ecuador, Filipinas, Guadalupe, Martinica y Camerún. El análisis de la secuencia del gen *Pfcyp51* que codifica la enzima 14α-desmetilasa CYP51 en 266 aislamientos mostraron una amplia gama de variaciones. Se describe también las inserciones de un elemento de 19 pares de bases (pb) que se descubrió en la región promotora del gen *Pfcyp51*. Se estableció la correlación entre los cambios en el *gen Pfcyp51* y su promotor con el aumento de la resistencia a los azoles. Además, se evaluó la contribución de las principales sustituciones en los aminoácidos de la CYP51 a través de la elucidación de 7 modelos computacionales de proteínas.

El **capítulo 4** describe por primera vez la identificación de un elemento de 19 pares de bases (pb) en la región promotora del gen *Pfcyp51*. La evidencia sugiere fuertemente que insertos de hasta 6 copias de este elemento en el promotor de cepas resistentes proporcionar sobre-expresión al gen en comparación con las cepas que contienen un elemento. Ensayos basados en PCR se utilizaron para analizar la presencia del elemento repetido en cuatro poblaciones de *P. fijiensis* de Costa Rica y en algunos aislados de Ecuador, África y el Sudeste Asiático. Experimentos de transformación de intercambio del promotor se utilizaron para analizar el papel de este elemento repetido en la expresión del gen *Pfcyp51*. Estos experimentos nos permitieron identificar a este nuevo elemento repetido como un componente que junto con las mutaciones en la región codificante del *Pfcyp51* son responsables de niveles superiores de resistencia contra los fungicidas azólicos.

El **capítulo 5** describe la generación de dos progenies F1 de *P. fijiensis* para la construcción de dos mapas genéticos basados en la resistencia a los fungicidas DMI. La tecnología DArTseq se utilizó para generar un mapa de ligamiento genético para ambas poblaciones. Se encontró total acuerdo entre los marcadores genéticos de ambas poblaciones, lo que subraya la solidez del enfoque. Esta herramienta genética fue esencial para identificar la región genética que determina la resistencia a los fungicidas DMI en la especie y apoya

firmemente la hipótesis de que el gen *cyp51* es el único importante determinante de la resistencia a fungicidas DMI en *P. fijiensis*. Esta región genética de 250.660 pb contiene 53 genes putativos que incluyen el gen *cyp51* que base en los hallazgos de otros autores y los nuestros es el candidato más plausible como la fuerza molecular que determina la resistencia a los fungicidas DMIs.

El **capítulo 6** analiza los resultados experimentales obtenidos en la tesis y los describe desde una perspectiva más amplia. Este capítulo resalta la evidencia convincente de que la modulación de la secuencia del promotor y la región codificante del gen *cyp51* se correlaciona con la perdida de sensibilidad observada en azoles. Finalmente, se discute el impacto y las implicaciones de estos hallazgos en las futuras estrategias para el control de enfermedades.

Samenvatting

Pseudocercospora fijiensis is de veroorzaker van black Sigatoka (of zwarte blad strepenziekte), de schadelijkste bladvlekkenziekte van het geslacht *Musa* dat ook bananen en bakbananen omvat. Vele soorten zijn vatbaar voor black Sigatoka, inclusief de "Cavendish" export variëteiten. Bladschade veroorzaakt belangrijke opbrengstverliezen en vroegtijdige afrijping van bananen, een belangrijke schadepost voor de exportindustrie. De belangrijkste beheersmethode voor black Sigatoka betreft het frequent bespuiten van plantages met fungiciden die een grote milieukundige en economische belasting vormen. Onder deze fungiciden omvat de chemische familie van de azolen de meest gebruikte werkzame stoffen om de ziekte te bestrijden. Azolen vallen onder de sterol demethylase remmers (DMIs) die het 14α-demethylase enzym (CYP51) blokkeren. Eén van de grootste problemen bij de bestrijding van black Sigatoka vormt de buitensporige en regelmatige toepassing van fungiciden op vele bananenplantages rondom de wereld. Het ongebreidelde gebruik van fungiciden heeft bijgedragen aan het ontstaan van populaties met een hoog niveau van DMI-resistentie. Gedurende de tijd is deze resistentie zodanig toegenomen dat het maximum aantal toepassingen in bereikt. Het verlies van gevoeligheid voor DMIs in *P. fijiensis* is gradueel ontstaan en dat geeft de indruk van een eigenschap die polygeen wordt gereguleerd. Desniettegenstaande blijkt in dit proefschrift dat het *Pfcyp51* gen uitsluitend verantwoordelijk is voor dit verlies onder veldomstandigheden. Onze studie omvat de eerste wereldwijde analyse van fungicideresistentie tegen DMIs in *P. fijiensis*, voorziet in een leidend principe om deze ontwikkeling te begrijpen, maakt het daarmee mogelijk om betere beheerstrategieën voor black Sigatoka te ontwerpen en roept op tot een duurzame oplossing voor deze ongeëvenaarde bedreiging van de bananenteelt.

In **Hoofdstuk 1** wordt het belang van banaan als fruit en voedselgewas beschreven alsmede het effect van black Sigatoka op de wereldwijde teelt van banaan en wordt het thema van dit proefschrift omschreven: fungicide resistentie tegen DMIs. Het beschrijft de levenscyclus en kenmerken van het pathogene organisme *P. fijiensis*, alsmede de geschiedenis van het gebruik van fungiciden en het effect van DMIs op natuurlijke populaties van de soort en wordt afgesloten met de opzet van het proefschrift.

In Hoofdstuk 2 wordt een historisch overzicht beschreven van de bestrijding van black Sigatoka – met name in Costa Rica - en welke strategieën daarbij werden ontwikkeld en ingezet. Het sluit af met een kritische analyse van deze praktijk en de gewenste veranderingen.

Hoofdstuk 3 beschrijft een uitvoerige wereldwijde phenotypering en genotypering van resistentie tegen DMIs in *P. fijiensis.* De gevoeligheid van 592 veldisolaten - afkomstig uit verschillende productiegebieden in Colombia, Costa Rica, de Dominicaanse Republiek, Ecuador, de Filippijnen, Guadeloupe, Martinique en Kameroen – tegen DMIs werd bepaald. Uit sequentieanalyses van het gen dat het 14α-demethylase enzym CYP51 codeert, *Pfcyp51,* in 266 isolaten komt een grote variatie naar voren. Daarbij werden ook inserties van een 19 bp fragment in de promotor van het gen gevonden en beschreven. De correlatie tussen deze veranderingen in de promotor en de toenemende resistentie tegen azolen was daarbij een opvallende constatering. Daarnaast werd het effect van de modulering van het CYP51 eiwit door diverse mutaties geëvalueerd door gebruik te maken van *in-silico* eiwitmodellen.

Hoofdstuk 4 beschrijft een *de-novo* identificatie van een 19 bp element dat werd gevonden in de promotor van het *Pfcyp51* gen. De meervoudige - tot zes kopieën - insertie van dit fragment in de promotor in resistente stammen leidt tot overexpressie van het *Pfcyp51* gen in vergelijking met isolaten die slecht één fragment in de promotor hebben. Een op PCR gebaseerde test werd gebruikt om de aanwezigheid van deze fragmenten in veldpopulaties uit Costa Rica en enige isolaten uit Ecuador, Afrika en Zuidoost Azië te onderzoeken. Transformatie experimenten waarbij de promotoren tussen gevoelige en resistente isolaten werden omgewisseld demonstreerden de rol van promotorinserties in de expressie van het *Pfcyp51* gen. Hiermee werd het geïnserteerde fragment als een nieuwe component van fungicideresistentie beschreven dat tezamen met mutaties in het coderende *Pfcyp51* gebied verantwoordelijk is voor de toenemende fungicideresistentie tegen azolen.

Hoofdstuk 5 beschrijft het maken van twee F_1 populaties van *P. fijiensis* die werden gebruikt om twee genetisch kaarten te maken van het gebied dat codeert voor DMI-fungicideresistentie en dat met DArT-technologie werd gekarteerd. Daarbij werd een volledige overeenkomst geconstateerd tussen de merkers in beide populaties die de robuustheid van de gehanteerde methoden onderstreepte. Deze benadering was essentieel om het gebied dat DMI-resistentie codeert in kaart te brengen en bevestigde de hypothese dat het *Pfcyp51* gen de bepalende factor is voor DMI-fungicideresistentie in *P. fijiensis.* Het gekarteerde gebied omvat 250,660 bp en bevat 53 mogelijke genen, waaronder *Pfcyp51,* als de belangrijkste kandidaat en de drijvende kracht achter de resistentie tegen DMIs in ons onderzoek en dat van anderen.

Hoofdstuk 6 is een algemene discussie over de uitkomsten van dit onderzoek en beschrijft deze in een breder kader. Hierbij wordt op overtuigende wijze aangetoond dat modulering van de promotor en het coderende gebied van *Pfcyp51* bepalend is voor en correleert met de waargenomen gevoeligheid voor azolen. Tenslotte worden het effect en de implicaties van dit onderzoek bediscussieerd ten aanzien van toekomstige beheersstrategieën van de ziekte.

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About the author

Pablo Antonio Chong Aguirre was born in 1977 in Guayaquil, Guayas, Ecuador. After completing high school in physics and mathematics in 1995, he began his studies at La Escuela Superior Politécnica del Litoral (ESPOL Polytechnic University) in Guayaquil and obtained his BSc degree in Marine Aquaculture in 2001 and his MSc degree in Biotechnology in 2007. In 2001 he started his research career as a junior researcher in the Plant Pathology department at the Centro de Investigaciones Biotecnológicas del Ecuador (CIBE, Ecuadorian Centre for Biotechnology Research), where he became head of the Molecular Biology department in 2007 and head of the Plant Pathology department from 2009 to 2012. In 2010 he was awarded a scholarship from the Secretaría de Educación Superior, Ciencia, Tecnología e Innovación (SENESCYT, Ministry of Science, Technology and Innovation) of the Ecuadorian government and a scholarship from ESPOL Polytechnic University. These allowed him to perform his PhD research abroad. In September 2012, he started his program in the group of Prof. Dr. Ir. Ing. Gert H.J. Kema at Wageningen University and Research, Plant Research International and the Laboratory of Phytopathology on the analysis of fungicide resistance in *Pseudocercospora fijiensis*, the causal agent of the banana black Sigatoka disease. This dissertation summarizes the research results of his PhD program on the origin, versatility and distribution of resistance mechanisms to DMI fungicides in *P. fijiensis*. After returning to Ecuador in 2016 he will continue his work as invited Professor of Biology and head of the Molecular Biology department at CIBE-ESPOL.

List of publications:

- An historical treatise and critical review of black Sigatoka control in banana production. Pablo Chong, Claudiana Carr, Gilberth Murillo, Mauricio Guzmán, Jorge Sandoval and Gerrit H.J. Kema. (to be submitted).

- Global analysis of reduced sensitivity to azole fungicides in the banana black Sigatoka pathogen *Pseudocercospora fijiensis*. Pablo Chong, Josué Ngando Essoh, Rafael Arango, L. C. Paul Keizer, Ioannis Stergiopoulos, Michael F. Seidl, Mauricio Guzman, Jorge Sandoval, Paul E. Verweij, Gabriel Scalliet, Helge Sierotzski, Luc de Lapeyre de Bellaire, Pedro W. Crous, Jean Carlier, Sandrine Cros, Harold J. G. Meijer, Esther Lilia Peralta1 and Gert H. J. Kema. (to be submitted).

- A new resistance mechanism to azole fungicides in the fungal banana black Sigatoka pathogen *Pseudocercospora fijiensis* is driven by increased expression of *Pfcyp51* through multiple promoter repeats. Caucasella Díaz-Trujillo*, Pablo Chong*, Viviane Cordovez, Mauricio Guzman, Pierre J.G.M. De Wit, Ioannis Stergiopoulos, Harold J. G. Meijer, Rafael E. Arango Isaza, Gabriel Scalliet, Helge Sierotzki, Esther Lilia Peralta and Gerrit H. J. Kema. (to be submitted).

- Genetic mapping of resistance to 14α-demethylase inhibitor fungicides in the banana black Sigatoka pathogen *Pseudocercospora fijiensis* reveals *Pfcyp51* as a single explanatory factor. Pablo Chong, Aikaterini Vichou, Henk Schouten, Harold J. G. Meijer, Rafael Arango and Gert H. J. Kema. (to be submitted).

- Brote de *Eacles imperialis* (Lepidóptera: *Saturniidae*) en Cultivos de Cacao y Frutales en Milagro – Ecuador Jorge Rafael Paredes Montero, Esther Lilia Peralta García, Pablo Antonio Chong Aguirre. Revista Tecnológica ESPOL – RTE, Vol. 23, N. 1, 13-19, (Diciembre, 2010).

- "Análisis del Polimorfismo de 21 Genotipos de *Musa spp*., Mediante el Uso de Marcadores Microsatélites" Christian Abraham Romero Bonifaz, Pablo Antonio Chong Aguirre Revista Tecnológica ESPOL – RTE, Vol. 22, N. 1, 1-6, (septiembre, 2009).

- "Diversidad Genética de Poblaciones de *Mycosphaerella fijiensis* Provenientes de Haciendas Bananeras con Manejo Orgánico y Convencional" Chong Pablo. Revista tecnológica de la ESPOL, Vol. 20, N. 1, 215 – 222, (octubre, 2007), ISSN: 0257-1749.

- Identificación de los genes Mat 1-1 y Mat 1-2 del hongo *Mycosphaerella fijiensis*, agente causal de la Sigatoka negra, para su cruce en laboratorio. Tesis de grado para la obtención del título de Biólogo al Sr. Julio A. Bonilla Jaime, Director de tesis: Pablo Chong Aguirre, Esther Lilia Peralta. Facultad de Ingeniería Marítimo y Ciencias del Mar, Escuela Superior Politécnica del Litoral. (22/05/2009)

- "Análisis Genético de la Resistencia A Triazoles En Aislados de *Mycosphaerella fijiensis* Para Poblaciones De Ecuador". Tesis de grado para la obtención del título de Bióloga a la Srta. Tatiana Paola Chávez Navarrete, Director de tesis: Pablo Chong Aguirre. Facultad de Ingeniería Marítimo y Ciencias del Mar, Escuela Superior Politécnica del Litoral. (22/06/2012).

*Equal contribution

Education Statement of the Graduate School

Experimental Plant Sciences

The Graduate School EXPERIMENTAL PLANT SCIENCES

Issued to: Pablo Antonio Chong Aguirre
Date: 31 October 2016
Group: Laboratory of Phytopathology & BU Biointeractions and Plant Health
University: Wageningen University & Research

1) Start-up phase	date
► **First presentation of your project**	
Mycosphaerella fijiensis population and fungicide resistance analyses	Oct 26, 2009
► **Writing or rewriting a project proposal**	
Fungicide Resistance in *Mycosphaerella fijiensis*	Jun 30, 2010
► **Writing a review or book chapter**	
► **MSc courses**	
► **Laboratory use of isotopes**	
Subtotal Start-up Phase	*4.5 credits**

2) Scientific Exposure	date
► **EPS PhD student days**	
EPS PhD Student day, Amsterdam University	Nov 31, 2012
EPS PhD Student day, Leiden University	Nov 29, 2013
► **EPS theme symposia**	
EPS Theme 2 symposium 'Interactions between Plants and Biotic Agents' and 'Willie Commelin Scholten Day', Amsterdam University	Feb 25, 2014
EPS Theme 4 symposium 'Genome Biology', Amsterdam University	Dec 14, 2015
► **NWO Lunteren days and other National Platforms**	
► **Seminars (series), workshops and symposia**	
ESPOL Ciencia 2010	Jan 21, 2010
29th Meeting of the Fusarium working group of the Koninklijke Nederlandse Planteziektenkundige vereniging	Oct 29, 2014
► **Seminar plus**	
► **International symposia and congresses**	
ACORBAT 2010, Medelin (Colombia)	Nov 08-12, 2010
PRIMER CONGRESO INTERNACIONAL DE BIOTECNOLOGÍA Y BIODIVERSIDAD (Ecuador)	May 28-31, 2012
17th Reinhardsbrunn Symposium on Modern Fungicides and Antifungal Compounds, Friedrichroda (Germany)	May 21-25, 2013
FRAC meeting 2014 (Germany)	Feb 04-05, 2014
► **Presentations**	
ESPOL Ciencia 2010 (Talk))	Jan 21, /2010
ACORBAT 2010 (Talk)	Nov 08, 2010
PRIMER CONGRESO INTERNACIONAL DE BIOTECNOLOGÍA Y BIODIVERSIDAD (Talk)	May 29, 2012
17th Reinhardsbrunn Symposium on Modern Fungicides and Antifungal Compounds (Talk)	May 21-25, 2013
FRAC meeting 2014 (Talk)	Feb 04-05, 2014
► **IAB interview**	
Meeting with a member of the International Advisory Board of EPS	Nov 14, 2012
► **Excursions**	
Subtotal Scientific Exposure	*12.0 credits**

3) In-Depth Studies	date
► **EPS courses or other PhD courses**	
Course Basic statistics	May 26,27, Jun 04,05,06, 2014
Course Bioinformatic	Aug 27-31, 2012
Course Transcription Factor and Transcriptional Regulation	Dec 17-19, 2013
Course Mixed model based genetic analysis in GenStat: from QTL mapping and association mapping to genomic prediction	Sep 02- 04, 2013
► **Journal club**	
Member of a literature discussion group at ESPOL	2010-2012
Member of a literature discussion group at PRI-WUR	2012-2014
► **Individual research training**	
Purdue USDA training	Apr-May 2011
Subtotal In-Depth Studies	*10.9 credits**

4) Personal development	date
► **Skill training courses**	
Course Techniques for Writing and Presenting a Scientific Paper	Dec 10-13, 2013
Course Project Management	Mar 19, Apr 02, 29, 2014
Course Scientific Writing	Apr 08- Jun 18, 2015
► **Organisation of PhD students day, course or conference**	
► **Membership of Board, Committee or PhD council**	
Subtotal Personal Development	*3.9 credits**

TOTAL NUMBER OF CREDIT POINTS*	**31.3**

Herewith the Graduate School declares that the PhD candidate has complied with the educational requirements set by the Educational Committee of EPS which comprises of a minimum total of 30 ECTS

** A credit represents a normative study load of 28 hours of study.*

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