# ECO-EVOLUTIONARY DYNAMICS OF DISEASE UNDER HUMAN-INDUCED SELECTION

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*For the ones to come.*

**Knowledge:** a tool from the old,
to understand the new.

# ABSTRACT

More than ten thousand years ago, humans started breeding plants as food supply: they chose those varieties of nutritional interest, grew them, and kept the seeds of the best plants for the next season. These practices were the beginning of agriculture, a long-term evolutionary experiment where humans act as a selective force. Active breeding is not the only way in which humans modify evolutionary trajectories: they also change the environment where species live. For example, global trade creates novel species interactions, and the urbanisation of wild areas alters ecological niches. Another compelling case of human-induced selection – and the topic of interest in this thesis – is the control of pathogens. Pathogens are regarded as a threat for human species survival, either because they are causing diseases in humans or because they constitute a risk to food security. In consequence, humans have developed management practices which intend to reduce or eradicate the population of these pathogens by applying abiotic (e.g. drugs) or biotic (e.g. biocontrol with other species) pressures. These strategies, as they deal with populations of living organisms, involve ecological and evolutionary processes. Thus, to improve pathogen control, we need to apply the current knowledge and techniques of ecology and evolution.

This thesis studies how pathogen populations are affected by the alternation of selective pressures to which they are exposed. Mainly, I study the dynamics of pathogen populations when host species are switched along time. The different reproductive rates of the pathogen in each host species can slow down the growth or diminish its population in the long-term. In agriculture, this can be achieved by using crop rotations in a field; in vector-borne diseases, the vector and the host are two different ecological niches for the pathogen, and the administration of drugs to the human host can be disadvantageous for pathogen reproduction in the vector. Using mathematical and computational models, I study host-pathogen interactions in infected crop fields and human populations affected by malaria. I simulate infections under multiple scenarios of selection in alternating host species and observe their progress or regression. The results are used to assess the optimality of human interventions for the control of the disease-causing pathogens. Overall, this thesis confirms that a better knowledge of eco-evolutionary principles in disease management can improve the design of strategies. This is especially true given the need for practices which are both efficient and sustainable across generations.

# KURZFASSUNG

Vor mehr als zehntausend Jahren begannen Menschen, Pflanzen als Nahrungsquelle zu züchten; sie wählten diejenigen Pflanzen aus, die zur Ernährung von Interesse waren, bauten sie an und behielten die Samen der besten Pflanzen für die nächste Saison. Diese Verfahren waren der Anfang der Landwirtschaft, ein langfristiges Evolutionsexperiment, in dem Menschen die Rolle der Selektion übernehmen. Bewusste Züchtung ist nicht die einzige Weise, in der Menschen den Verlauf der Evolution verändert haben: sie verändern auch die Umwelten, in denen Spezies leben. Zum Beispiel hat der globale Handel neue Interaktionen zwischen Spezies geschaffen, und die Urbanisierung wilder Lebensräume hat ökologische Nischen verändert. Ein anderer klarer Fall von durch Menschen hervorgerufener Evolution – und das ist das Thema dieser Arbeit – ist die Kontrolle von Pathogenen. Pathogene werden als Bedrohung für das überleben der Menschheit gesehen, entweder weil sie Krankheiten in Menschen verursachen oder weil sie ein Risiko für eine ausreichende Lebensmittelversorgung darstellen. Deshalb haben Menschen Management-Verfahren entwickelt mit dem Ziel, die Populationen dieser Pathogene zu reduzieren oder zu eliminieren, entweder durch den Einsatz abiotischer (z.B. Medikamente) oder biotischer (z.B. biologische Kontrolle durch andere Spezies) Mittel. Da diese Strategien auf lebendige Populationen angewandt werden, triggern sie ökologische und evolutionäre Prozesse. Daher müssen wir, um Pathogene besser kontrollieren zu können, das bestehende Wissen und die bestehenden Methoden aus ökologie und Evolution nutzen.

Die vorliegende Arbeit untersucht, wie Pathogen-Populationen durch wechselnden Selektionsdruck beeinflusst werden, denen sie ausgesetzt werden. Ich untersuche hauptsächlich die Dynamik, wenn sich die Wirtsspezies über die Zeit ändert. Die unterschiedlichen Reproduktionsraten des Pathogens in den verschiedenen Wirtsspezies kann das Wachstum verlangsamen oder langfristig die Population verkleinern. In der Landwirtschaft kann das durch geeignete Fruchtfolgen oder -wechsel erreicht werden; in durch Vektoren übertragenen Krankheiten stellen der Vektor und der Wirt zwei verschiedene ökologische Nischen für den Erreger dar, und die Verabreichung von Medikamenten an den menschlichen Wirt kann die Vermehrung des Pathogens im Vektor negativ beeinflussen. Unter Verwendung von mathematischen und Computer-Modellen untersuche ich die Interaktionen zwischen Pathogen und Wirt in von Krankheiten befallenen Nutzpflanzen und in menschlichen Populationen, die mit Malaria infiziert sind. Ich simuliere Infektionen in einer Reihe von Szenarien, in denen sich der Selektionsdruck durch sich abwech-

selnde Wirtsarten ändert, und beobachte ihr Fortschreiten oder Abklingen. Die Ergebnisse dienen dazu, zu bewerten, welche menschlichen Eingriffe für die Kontrolle von Krankheitserregern optimal sind. Insgesamt bestätigt diese Doktorarbeit, dass eine bessere Kenntnis von öko-evolutionären Prinzipien im Management von Krankheiten das Design von Strategien verbessern kann. Dies gilt insbesondere in Anbetracht der Notwendigkeit von Verfahren, die sowohl effizient sind als auch über mehrere Reproduktionszyklen hinweg nachhaltig sind.

# CONTENTS

Part I

# GENERAL INTRODUCTION

# 1

# THESIS BACKGROUND, SCOPE AND OVERVIEW

In his pivotal book *On the origin of species*, Darwin pointed out that humans perform selective breeding for desirable characteristics among individuals, a process also referred to as **artificial selection** (Darwin, 1859). In contrast to natural selection, in artificial selection, humans are the agent that determines the forms of another species that succeed to the next generation. We have clear examples of artificial selection in **domesticatory relationships**, where the domesticator – e.g. human – influences the reproduction of the domesticate – e.g. animals, fungi or plants – with advantages for both organisms (Zeder, 2015).

We can also find human-induced selection in practices which involve the manipulation of the growth conditions of biotic agents. **Disease management strategies** are a relevant example, where we aim to decrease the abundance of the **pathogen**, the disease-causing agent, in benefit of the infected host organism. In contrast to domestication, the object of evolution is not selected through reproduction directly, but through a change in its **environment**; in disease, the host (Rynkiewicz et al., 2015). Moreover, the goal is often the eradication of a population, instead of an increased reproduction.

In both scenarios, human action impacts an organism **evolution**. Humans apply a (direct or indirect) selective pressure which determines the reproductive success, or **fitness** (Day and Otto, 2001). If there is genetic variation among the individuals affected, the consequence of the action is a change of allelic frequencies in the population. This process is combined with a change of interactions between individuals and their biotic and abiotic environment, i.e. **ecology**. The perturbation can involve, for example, population size variations that feedback into the evolutionary trajectory, and vice-versa, in coupled **eco-evolutionary dynamics** (Fussmann et al., 2007).

This thesis is the result of a broad aim to study the **eco-evolutionary principles of disease management strategies** using mathematical and computational methods. Using example cases in agriculture and biomedicine, I show how theoretical models can help to understand the processes underlying pathogen infection success and the consequences of human activity on them. Particularly, I focus on the use of host differential selection as a tool to improve infection control, through the design of eco-evolutionary informed strategies for pathogen management.

## 1.1 THE INTERPLAY BETWEEN ECOLOGY AND EVOLUTION

Back in 1866, Haeckel defined *Ökologie* as "the relation of the animal both to its organic as well as its inorganic environment" (Haeckel, 1866). This was only a few years after the publication of the natural selection theory by Darwin and Wallace, in 1859 (Darwin, 1859; Wallace, 1858). Although these cornerstones of ecology and evolution were contemporary, the two disciplines were studied separately until the early 20th century. First, the focus was on the influence that ecological interactions had on evolutionary mechanisms: a mismatch of time-scales would make ecology relevant for evolution but not vice-versa. Recently, bidirectional feedback has been acknowledged, coining modern eco-evolutionary dynamics (Pelletier et al., 2009; Schoener, 2011).

Nowadays, the knowledge on eco-evolutionary feedbacks is increasing from both experimental (Hendry, 2019) and theoretical (Govaert et al., 2019) approaches. The focus is not exclusively on two-species interactions, but also on multispecies and community dynamics (Becks et al., 2012; Frickel et al., 2017) , at molecular level, population level or across scales (Brunner et al., 2019). Moreover, applications of the eco-evolutionary approach are being expanded to questions related to evolution of life-history traits (Cameron et al., 2013) , evolution of cooperation (Gokhale and Hauert, 2016) or cancer research (Gatenby and Brown, 2018).

The key question of the field, as proposed by Gregor Fussmann, would be "What reciprocal effect do ecological and evolutionary dynamics have on each other over ecologically relevant time-scales?" (Fussmann et al., 2007). Starting from evolution, variations in genotypes and their frequencies change phenotypic traits. If these traits are relevant for the interaction between organisms, the dynamics of populations, communities and ecosystems are altered. From an ecological point of view, the abundance of a genotypic or phenotypic variant can determine the fitness of a trait, as in frequency-dependent selection (Ayala and Campbell, 1974).

To illustrate, we can focus on predator-pray interactions. The predator species can consume different types of prey individuals, but the most common will often be the prey of choice, which provides a reproductive advantage to the rare types (Allen, 1988). In its turn, this will provoke rare varieties to turn more popular, and the predator species might shift their choice, or other variant of predator species will start catching them, turning into antagonistic coevolution (Van Valen, 1973). This phenomena is analogue to the competition in host-pathogen interactions, which characteristics determine the success of the infection (see Box 1).

## 1.2 HOST-PATHOGEN INTERACTIONS IN WILD AND DOMESTICATED ECOSYSTEMS

Similarly to predator-prey interactions, the evolution of hosts and their pathogens is co-dependent: one species imposes a selective pressure on the other. The species competition works as the force which maintains genetic polymorphisms in both organisms. However, the pace at which traits change is different in wild and domesticated ecosystems; in the wild, both interacting species are the object of natural selection while in scenarios of domestication human-induced selection acts purposedly on the host. Because of the artificial selection, the term coevolution is often avoided for pathogen interactions with domesticated hosts.

In wild ecosystems, hosts and pathogens follow antagonistic coevolution: the increase in fitness of one is detrimental to the other, which needs to evolve in turn. The constant evolution caused by interspecies competition is referred as the Red Queen hypothesis (Van Valen, 1973). When observing the changes in allele frequencies, we can find two general patterns: selective sweeps and dynamic polymorphisms. In the first, new beneficial alleles emerge and fixate in the population by recurrent selective sweeps; selection is directional and genetic change accumulates in both populations. This pattern is often understood in analogy to arms races, in which both populations 'improve' their mechanisms (Dawkins and Krebs, 1979). In the second, there are fluctuations in allele frequencies; evolution is non-directional and the existing genetic variation oscillates in time (Woolhouse et al., 2002).

In human-domesticated ecosystems, humans encourage preferentially the reproduction of one variety of host, creating uniformity in the selection pressure for the pathogen. Then, strong directional selection acts and pathogens evolve more quickly than in heterogeneous wild ecosystems that impose a weaker, disruptive selection (McDonald and Linde, 2002). Moreover, host homogeneity increases pathogen transmission and density, which lowers its exposure to genetic drift effects and increases the effective population size (Stukenbrock and McDonald, 2008).

The evolution between hosts and pathogens also plays a role when humans provoke changes to their natural ecosystems, out of domesticatory relationships. A well-documented example is the human introduction of a virulent myxoma strain in Australia to reduce the population of European rabbits (Fenner and Fantini, 1999; Kerr, 2012). European rabbits were brought to Australia in 1788 by English settlers as a food source, but they became feral and overgrew. As biological control, highly lethal viruses were introduced, but rapid selection favoured less virulent strains which enhanced disease transmission. When rabbits with some resistance became

more abundant, viral strains increased virulence. Evolution changed the expected population dynamics, which resulted in reduced effectiveness of the control measure.

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**Box 1. Characteristics of host-pathogen interaction determining the infection success: infectivity, virulence, resistance, tolerance.**

Pathogens establish parasitic relationships with their hosts and cause disease. The severity of the epidemic depends on the relation between hosts, pathogens and their environment (Scholthof, 2007). Here we focus on pathogen infectivity and virulence, and host resistance and tolerance as characteristics that define the host-pathogen interaction.

Infection requires molecular and physical mechanisms that allow the establishment of the pathogen into the host. The ability of a pathogen to establish infection is named **infectivity**. Once infected, the pathogen provokes damage to the host. The exploitation of host resources can cause minor structural alterations or severe affectations that lead to host death. The amount of potential damage is referred to as **virulence** (Gandon et al., 2002).

On the host side, **resistance** refers to the ability of the host to limit pathogen burden. Mechanisms that provide resistance can prevent the infection from happening or can prevent pathogen growth once the infection has occurred. Resistance differs from host **tolerance**, which is the ability to permit the presence of the pathogen without limiting its burden. Tolerance mechanisms control tissue damage caused by pathogen-derived virulence factors or the immune response (Glass, 2012).

When speaking about mechanisms of infection, infectivity and virulence are sometimes used inter-changeably. Notably, in plant pathology, the gene-for-gene model of infection denominates avirulent the pathogen that is unable to establish an infection and virulent the pathogen that can establish infection (Flor, 1956).

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## 1.3 THE ROLE OF HOST HETEROGENEITY

In both natural and domesticated ecosystems, pathogens are generally at an advantage relative to their hosts because they have a shorter generation time and larger population sizes, which allows for more mutations in a fixed period of time (Thomas et al., 2010; Zhan et al., 2014). Hosts can contrarrest through sexual reproduction (Hamilton et al., 1990), but also with spatial and temporal heterogeneity. Heterogeneity forces pathogens to invest resources in life-history traits such as propagule

production, dispersal and transmission, which are a trade-off for increased infectivity or virulence (Thrall and Burdon, 2003; Zhan et al., 2014).

In anthropogenic ecosystems, host heterogeneity can be provided by spatial variation, temporal cycling or mixing of selective pressures (Carroll et al., 2014). The goal of spatial variation is to protect susceptible forms, by combining them with resistance ones. For example, mosaics of resistant crops within a field in agriculture (Sapoukhina et al., 2009). Temporal variation relies on the alternation of treatments, so the pathogens adapted to a single one face disadvantages when the environment is switched. This can be encountered in crop rotation (Marcroft et al., 2012) or sequential antibiotic treatments (Roemhild and Schulenburg, 2019). When mixing, pathogens face a diversified selection: they encounter multiple stressors simultaneously. Examples could be pyramiding of resistance genes in crops (Pedersen and Leath, 1988) or combined drug therapies (Saputra et al., 2018). All these examples, noticeably, involve human action directed to pathogen control.

The lack of host heterogeneity has the adverse effect, facilitating pathogen spread and transmission. It is exemplified in the Panama disease of bananas (Ploetz, 2000). By 1950s, most of the world's commercial bananas depended on a single cultivar: the Gros Michel. This variety was susceptible to a wilt caused by the fungus *Fusarium oxysporum f.sp. cubense*. In 1950, a first outbreak of the wilt in Panama spread worldwide and the banana industry got in crisis due to its severity. The industry recovered when Gros Michel plantations were substituted by the Cavendish cultivar, resistant to the wilt. Nowadays, banana cultivation faces a similar threat as plantations still rely on a single variety: a newly discovered *F. oxysporum* strain with virulence for the Cavendish is spreading through the globe (Ploetz, 2015).

## 1.4 HUMAN ACTION IN ECOSYSTEMS: MANAGING DISEASE

Human activities have an impact on the global biotic and abiotic environment. Global travel creates novel species associations; urbanisation or agriculture change density and diversity of hosts, parasites and their vectors; and interventions such as culling can result in unintended evolutionary pressures. These activities affect host and parasite traits and lead to increased frequency and severity of emergent infectious diseases (Rogalski et al., 2017). Nonetheless, human action can also prevent, reduce or eradicate diseases through the use of management strategies.

Disease management programs identify vulnerable points in the disease cycle and apply tactics directed to prevention of the infection (prophylaxis) or curation of the infected host (therapy). In the design of strategies, the interaction between three factors is crucial: host, infectious (or abiotic) agent, and the environment, also con-

ceptualised as the disease triangle (McNew, 1960; Scholthof, 2007). As a generic example, a microorganism can be pathogenic for a plant (host-pathogen interaction), but might exclusively grow and successfully infect when there are high levels of relative humidity (host-pathogen-environment interaction). Disease transmission between hosts is also important, as when the pathogen uses an intermediate vector organism, targeting the vector can also be effective.

Not only host-pathogen-environment interactions modify the disease outcome, but also the evolution of all the agents involved. Current strategies in translational evolutionary biology tackle disease control as a global challenge, especially, regarding human health and food security (Carroll et al., 2014). Evolutionary-based strategies, then, are designed to act at different levels. For example, in crop breeding we can identify traits for pathogen resistance in cultivated varieties which can be hybridised; and complement it with the synthetic engineering of specific crop genes (Varshney et al., 2011). In parallel, at the systemic scale, providing an heterogeneous environment can slow down pathogen adaptation.

Thus, the application of evolutionary principles is essential, specially considering therapy failure due to pathogen evolution of resistance against antimicrobial agents (e.g. drugs or fungicides). An integration of techniques that consider, for example, host heterogeneity with other traditional criteria could lead to successful disease control both in the short and long term. The effects, and potential synergies, are under research in both the biomedical and agriculture fields.

## 1.5 THEORETICAL MODELS FOR EVOLUTIONARY DISEASE MANAGEMENT

Research in ecology and evolution has historically relied on mathematical models to describe population dynamics (Bacaer, 2011). We can find early examples in the Malthusian growth (Malthus, 1798); the growth bounded by resource limitations in the Verhulst's logistic equation (Verhulst, 1838); or the population genetics models developed by Fisher, Haldane and Wright during the modern synthesis (Fisher, 1930; Haldane, 1927; Wright, 1930).

Relevantly, two-species interactions were formalised in Lotka-Volterra predator-prey equations (Lotka, 1920; Volterra, 1928), which were used later on for host-pathogen coevolution, as both systems consisted of two competitive oscillatory species. On the other hand, models for disease dynamics root in the Ross model of malaria transmission by mosquitoes (Ross, 1910); and McKendrick and Kermack's model of susceptible-infected-resistant host epidemiology (Kermack and McKendrick, 1927).

Therefore, theoretical biology has provided a valuable tool to study disease based on the population dynamics of the agents involved. Current disease models are used to understand the spread and severity of the epidemic, to predict its outcome and to inform intervention strategies. They can focus on the individual scale, e.g. studying within host competition of pathogen strains (Bushman et al., 2018), or take into account the whole population, e.g. considering the effects of herd immunity (Milne et al., 2015). In all cases, theoretical models permit the in-silico testing of multiple hypotheses without time or resource constraints.

While the development of analytical theory with proof-of-concepts models can be itself a test of verbal hypotheses (Servedio et al., 2014), applied theoretical models can help the understanding of biological processes and predict the dynamics of populations in predefined scenarios. However, knowing the use and the assumptions underlying a model is fundamental for the correct interpretation of its results. This has sometimes led to the devaluation of models in contrast to experimental evidence (Goldstein, 2018). Notwithstanding, theory and experiments are two complementary approaches which should feed and learn from each other.

## 1.6 THESIS SCOPE AND OVERVIEW

The objective of this thesis is to **assess the optimality of disease management strategies that integrate host-pathogen eco-evolutionary dynamics**, given specific goals of food security and human health.

For the study, I focus on strategies that control pathogens by a **temporal variation** in selection. The premise is that switching selection pressures slows the growth and modifies the evolution of the pathogen. In this case, variation of selection pressures is imposed by rotation of hosts in scenarios related to **agriculture**, for food security, and **biomedicine**, for human health.

- In agriculture, I consider a pathogen which has different infectivity for different crop types in a crop rotation setting. The aim is to control the pathogen to avoid a reduction in the seasonal crop yield, using cultural, genetic and/or chemical control. The long-term maximisation of yield is required for food security.
- In biomedicine, I study *Plasmodium*, the pathogen that causes malaria, which naturally switches from host to vector (as alternative host) in its life cycle. I focus on the effect that an external abiotic stressor (drug) has on the transmission of the pathogen between populations. Given the worldwide impact of malaria, studying scenarios of possible transmission interruption has important implications for global human health.

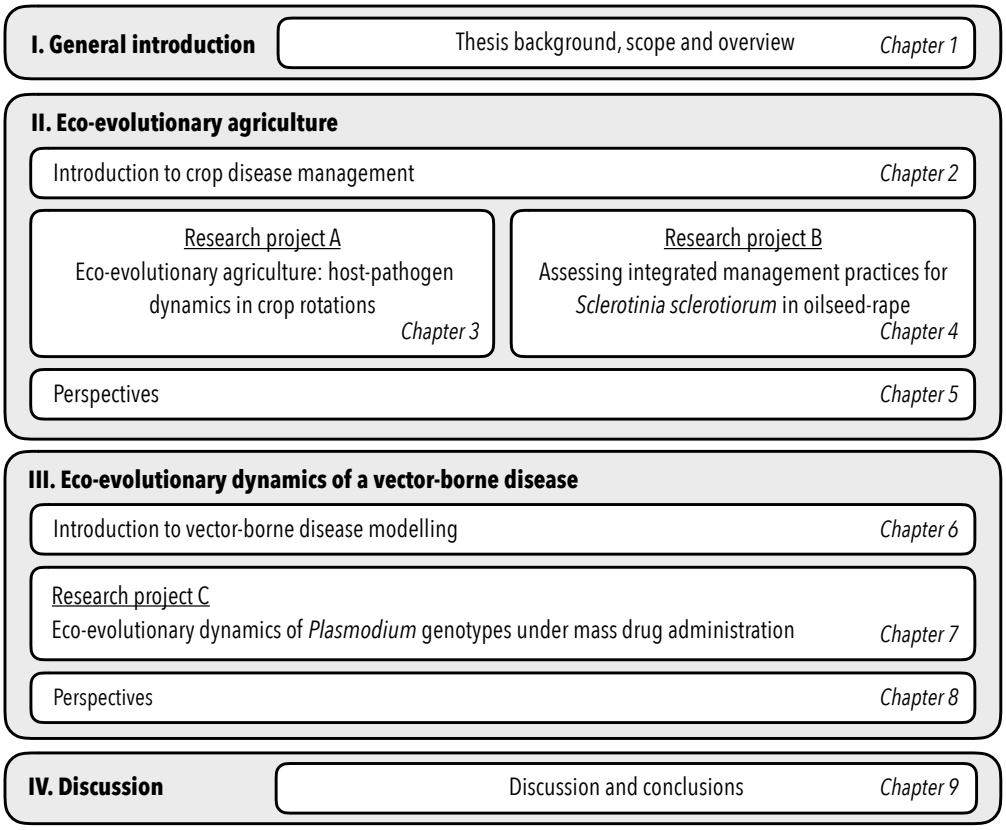


Figure 1.1: **Thesis overview.** The thesis is divided in four parts (grey boxes) which contain a total of nine chapters (white boxes).

In total, the thesis has four parts, including the current introduction (Part I). The intermediate parts correspond to research in agriculture (Part II) and research in biomedicine (Part III). Within these two parts, there is a total of three research projects (Chapters 3, 4 and 7). The thesis finishes with a general discussion (Part IV) (Fig. 1.1).

*Summary of the research projects*

In Part II, entitled 'Eco-evolutionary agriculture', I introduce pest control in agriculture alternating different cropping strategies through two models:

- First, I present a generic model of crop rotations of host and non-host crops. I consider agronomic variables of soil quality and cash yield to optimise the rotations in absence of pathogen. Then, I compare the rotation patterns with the ones obtained with pathogen infection. I implement host-pathogen dynamics and compare the ecological vs. eco-evolutionary dynamics by considering pathogen strains with a higher virulence. Finally, I analyse the repetition of rotation patterns in the long term.
- For the second model, I apply the generic framework to the case study of the white mould of oilseed rape, *Sclerotinia sclerotiorum*, and its host, *Brassica*

*napus*. In this model, I extend the strategies to include a resistant cultivar of oilseed rape and the application of fungicides. A spore bank that serves as reservoir for the fungi is modelled, as well as seasonal yield. Strategies are evaluated according to their ability to control infection and maximise healthy oilseed rape yield.

I finish this part including perspectives on the application of the models. These perspectives concern the evolution of pathogen virulence to resistant host crops and the application of optimisation algorithms to explore large sets of possible strategies according to their economic balance of benefits and costs.

In Part III, entitled 'Eco-evolutionary dynamics of a vector-borne disease', I focus on malaria, as an example of a widespread disease which parasite goes through different selection pressures during its life-cycle in the host and the vector.

- In the model, I use a compartmentalised *Plasmodium* life-cycle to study the parasite dynamics in isolated human populations under antimalarial mass drug administration. The chosen drugs are atovaquone and chloroquine, as for both there is experimental evidence of reproductive disadvantage for parasite strains carrying drug resistance. The parasite genotype frequencies are tracked during consecutive events of disease transmission, observing transmission interruption in some treatment scenarios.

This part also discusses future directions of the model applicability.

A list of publications related to the thesis and the detailed author contributions for each section can be found at the end of the document (see 'List of papers, manuscripts and contributions').

# Part II

# ECO-EVOLUTIONARY AGRICULTURE

# 2

# INTRODUCTION TO CROP DISEASE MANAGEMENT

For thousands of years, humans have conducted an extensive artificial selection experiment: agriculture. However, the process has been continuously challenged by evolving plant pathogens, which have threatened the maximisation of crop yield and imposed non-sustainable control methods. This section highlights the use of ecological and evolutionary theory when incorporated in current agricultural practices, as a path to alternative solutions. The strategies that we propose are a mix of the conventional crop rotation patterns informed by pathogen population dynamics as well as an introduction of resistant crop types and fungicides. How these strategies affect pathogen evolution is also a question of study here. Eco-evolutionary agriculture, as presented, can be crucial to the design of sustainable farming, needed in the face of the global challenge of food security.

## 2.1 CONCEPTUAL FRAMEWORK

*The man-guided evolution of rusts and other insights on eco-evolutionary cropping*

In the paper "Man-Guided evolution in plant rusts", Johnson (1961) discussed that selective crop breeding does modify not only the host plant but also the pathogen. He studied cereal rusts and described the pathogen response to the growth of crop varieties with resistance genes. In the response, a mutated pathogen with virulence for the specific host gene of resistance would spread, farmers would respond by introducing a new single-gene resistant cultivar – nowadays a major resistance R gene (Hammond-Kosack and Kanyuka, 2007)–, and the process would repeat, in a boom-and-bust cycle. Although the paper focused on pathogen evolution of virulence, it reflects that cropping decisions affect plant pathogens, both from the ecological and evolutionary perspectives.

The use of genetic resistance cultivars is, together with the application of chemicals (e.g. fungicides) the most popular strategy for pathogen control in agriculture. However, the rapid evolution of pathogens requires insights which do not only act on the short term (as in the use of single R genes) but also in the long run. More prolonged effects have been reported on strategies for deployment of multiple resistance genes within a single cultivar – pyramiding (Pedersen and Leath, 1988) – or within a field in regional mosaics (Sapoukhina et al., 2009), the second often with a better performance (Djidjou-Demasse et al., 2017). However, heterogenicity can

also be achieved temporally through the cycling of hosts. In this case, cultural control has played an essential role during centuries, by farmers practising seasonal rotations of crops in their fields (Bruns, 2012; Marcroft et al., 2012).

*The use of crop rotations*

Early farmers started using crop rotations in their fields because of the improved seasonal outcome. Alternating crops became crucial for overcoming problems associated with soil nutrient depletion, but also for diminishing damages caused by pests. Three-field rotation was used from the Middle Ages until Townshend culminated the popularity of four-crops rotation system in the 18th century (Bruns, 2012; Wigelsworth, 2006). Later on, during the Agricultural Revolution of the mid-20th century, rotation systems were modified to include soil chemical supplements or further advanced practices (Paarlberg and Paarlberg, 2000).

Farmers cultivate diverse crops with different purposes. Cereals such as wheat, barley or corn provide a product to be commercialised, whereas other crop variants such as clover, vetch or cowpea help fixing nitrogen or incrementing aeration of the field soil (Mohler and Johnson, 2009). Usually, these variants are alternated in regular patterns of three or four seasons, obtaining a higher yield outcome for the commercial crops. From the epidemiology side, some crop species are non-hosts for pathogens that affect host crops of interest. In the design of rotations, the non-hosts are cultivated alternatively as break crops, which 'break' (as stop or prevent) the epidemic from developing further (Angus et al., 2015). The pathogens' struggle to reproduce in the absence of host has lead, in some cases, to the evolution of inter-season survival traits – such as soil persistence structures – or the adaptation to weedy species, used as green bridges between seasons (Zhan et al., 2014). Despite this fact, the reduction of pathogen load that break crops provide is valuable and is widely used as cultural control of crop pathogens.

Further improvement of disease control by crop rotations would require complementation with other techniques, such as genetic resistance or the use of fungicides. We would speak, then, about integrated pest management (IPM) (Kogan, 1998; Smith and Reynolds, 1966), which by definition uses all suitable techniques – genetic, chemical, biological control – to maintain pathogen population levels below those causing economic losses.

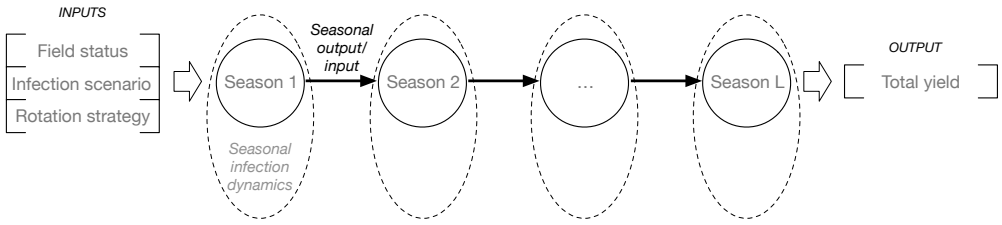


Figure 2.1: **Diagram of the model framework for eco-evolutionary agriculture.** Before the simulation is run, the values of parameters and variables that characterise field status, infection scenario and rotation strategy are defined and given as input. Then, seasonal infection dynamics are simulated for $L$ consecutive seasons, modifying the values of the variables: the output of one season serves as input for the next one. When the simulation ends, we have a final output which corresponds to the total yield, a variable of interest.

## 2.2 METHODOLOGICAL FRAMEWORK

Here, I present a model to study temporal rotations of crops within a single field. The main features that define it are the following:

- It combines processes happening within seasons (in continous time) and between seasons (discrete time).
- It models infection dynamics within the Lotka-Volterra framework (Lotka, 1920; Volterra, 1928).
- It predefines the interaction of one or multiple pathogens with multiple hosts types using a pathogen fitness matrix.
- It tracks variables of agronomic interest.

In the diagram (Fig. 2.1), variables and parameters referring to the field status, infection scenario and rotation strategy are given as inputs to the first season. Within the season, infection dynamics occur. The seasonal output is received as input for the second season, where within season dynamics occur again. After the last season, total yield is given as output of the simulated strategy.

### *An hybrid dynamical system for seasonal dynamics and harvest*

To implement the infection dynamics through a temporal variation of host crops, or rotation, we need to work at two different time scales: phenomena happening within a season, in a continuum of time, and phenomena happening between seasons, in discrete units of time. Because of this combination, the model falls into the category of hybrid dynamical systems.

Hybrid dynamical systems (HDS) are defined as dynamical systems whose evolution depends on a coupling between variables that take values in a continuum and variables that take values in a finite or countable set (Schaft and Schumacher, 2000). Considering HDS, our model can also be regarded as a hybrid automaton, in which the discrete variables work following a finite state machine, in which each state depends on a finite set of continuous variables, described by ordinary differential equations (ODEs). In our system, we combine discrete variables which are updated after every harvest, following recursive equations of the form:

$$n(t+1) \quad = \quad n(t) + increase - decrease, \tag{2.1}$$

Which contrast with the continuous variables used in infection dynamics, that follow differential equations of the form:

$$\dot{n} \quad = \quad rate\ of\ increase - rate\ of\ decrease. \tag{2.2}$$

*Lotka-Volterra equations for host-pathogen dynamics*

Disease dynamics can be modelled with a focus on the host infection status – as in the susceptible-infected-resistant (SIR) epidemiological framework (Kermack and McKendrick, 1927) – or via host-pathogen explicit dynamics. To develop our model, we focus on the second using a modification of the Lotka-Volterra equations, widely used in theoretical ecology (Lotka, 1920; Volterra, 1928).

The Lotka-Volterra competitive equations represent the coevolutionary dynamics of a prey-predator population, by reflecting the change of density of the competing species along time. They are two first-order, nonlinear, differential equations, described as follows:

$$\dot{x} \quad = \quad \alpha x - \beta xy \tag{2.3}$$

$$\dot{y} \quad = \quad \gamma xy - \delta y \tag{2.4}$$

where $x$ and $y$ are the variables corresponding to the prey and predator population, respectively. Because they are differential equations, the change happens in continuous time and depends on the balance of the growth of the population (positive signed terms) and the decay of the population (negative signed terms). In the generic case, prey $x$ grows at a rate $\alpha$ ($\alpha x$), and its death depends on the prey and predator meeting ($\beta xy$) and the rate of predation $\beta$ (Eq. 2.3). The growth of the predator $y$ depends on meeting the prey ($\gamma xy$) at a rate of prey consumption $\gamma$ and its intrinsic death ($\delta y$) (Eq. 2.4). The rates $\alpha, \beta, \gamma, \delta$ are constant parameters.

In the analogy between prey-predator and host-pathogen, the variables correspond to the host ($x$) and the pathogen ($y$) densities. The rates correspond to host

growth ($\alpha$), host tolerance or pathogen virulence ($\beta$), pathogen infectivity ($\gamma$), and pathogen death ($\delta$).

*Pathogen fitness matrix for characterising pathogen-host interactions*

In the generic Lotka-Volterra equations presented, there is one host and one pathogen species, but the extension to multiple species or species types is possible. To characterise the interaction of each pair, we use a matrix describing the compatibility of each pathogen strain ($p_j$) for each host ($h_i$), for a population of $n$ pathogens and $m$ hosts where $j$ and $i$ indicate the pathogen and host index, respectively (Eq. 2.5). This matrix is later coupled with the equation terms which involve a meeting of the pathogen with the host,

$$W_{ji} = \begin{array}{c} \\ p_0 \\ p_1 \\ \vdots \\ p_{j=n} \end{array} \begin{array}{c} \begin{array}{cccc} h_0 & h_1 & \dots & h_{i=m} \end{array} \\ \begin{pmatrix} w_{00} & w_{01} & \dots & w_{0m} \\ w_{10} & w_{11} & \dots & w_{1m} \\ \vdots & \vdots & \ddots & \vdots \\ w_{n0} & w_{n1} & \dots & w_{nm} \end{pmatrix} \end{array}. \tag{2.5}$$

In the matrix, values of $w_{ij}$=1 maintain the original rate values; values of $w_{ij} \geq 1$ reinforce the interaction, $w_{ij} \leq 1$ weaken the interaction, and values of $w_{ij}$=0 make the interaction null. Because these interactions modify pathogen growth – or reproduction – we refer to the matrix as pathogen fitness matrix.

## 2.3 RESEARCH QUESTIONS

I use the methodological framework presented to study two research questions:

- Given a field where cash crops and cover crops can be alternated; which rotation patterns maximise yield under an infection which affects cash crops, but not cover crops?
- *Sclerotinia sclerotiorum* is a fungus that affects oilseed rape, *Brassica napus*, among other crops. When oilseed rape is cultivated in rotation, how many seasons of break crop are needed to prevent a build-up of the infection? Can we reduce this number when genetic resistant cultivars or fungicides are applied?

The questions are developed as two different studies, presented as manuscripts in Chapter 3 and Chapter 4, respectively.

# 3

# ECO-EVOLUTIONARY AGRICULTURE: HOST-PATHOGEN DYNAMICS IN CROP ROTATIONS

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The content of this chapter has been peer-reviewed and it is published as:

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The `Python` core codes, describing the model, are available on `Github` at *https://github.com/tecoevo/agriculture*.

## 3.1 ABSTRACT

Since its origins, thousands of years ago, agriculture has been challenged by the presence of evolving plant pathogens. Temporal rotations of host and non-host crops have helped farmers to control epidemics among other utilities, but further efforts for strategy assessment are needed. Here, we present a methodology for developing crop rotation strategies optimal for control of pathogens informed by numerical simulations of eco-evolutionary dynamics in one field. This approach can integrate agronomic criteria used in crop rotations – soil quality and cash yield – and the analysis of pathogen evolution in systems where hosts are artificially selected. Our analysis shows which rotation patterns perform better in maximising crop yield when an unspecified infection occurs, with yield being dependent on both soil quality and the strength of the epidemic. Importantly, the use of non-host crops, which both improve soil quality and control the epidemic results in similar rational rotation strategies for diverse agronomic and infection conditions. We test the repeatability of the best rotation patterns over multiple decades, an essential end-user goal. Our results provide sustainable strategies for optimal resource investment for increased food production and lead to further insights into the minimisation of pesticide use in a society demanding ever more efficient agriculture.

## 3.2 AUTHOR SUMMARY

The invention of agriculture is a major evolutionary transition in the social evolution of the human race. Transforming the lifestyle from nomadic to sedentary, agriculture provided humankind with the stability necessary to make rapid advancements. However, agriculture, as we know it, is now in danger. While agriculture is a grand arti-

ficial selection experiment, it is in a constant battle with the brute force of natural selection, generating highly infectious plant pathogens. Traditional techniques such as slash-and-burn techniques are not sustainable for feeding the ever-increasing population. Crop rotation, on the other hand, has been developed over thousands of years as a sustainable method. We provide a computational model of how crop rotations can be used to tackle pathogen infection and what properties of rotation patterns make them sustainable in the long run. We hope that this study, together with other sustainable methods such as minimal pesticide use and biocontrol, can make agriculture more efficient.

## 3.3 INTRODUCTION

Around ten thousand years ago, changes in climate conditions led to the emergence of agricultural practices in human hunter-gatherer communities around the globe (Evans, 1999). This process of domestication – or artificial selection – was refined along centuries through trial and error, combined with experience, increasing the quantity and quality of the product. In the case of plant agriculture, the presence of pests has been a substantial threat to effective production (Oerke, 2006). The first farmers already tried to overcome the pest problem by employing field rotations, i.e., shifting cultivation techniques (Bullock, 1992; Mohler and Johnson, 2009; Nickel, 1973), among other methods. As the human population continues to multiply, current agriculture practices need to address a two-fold problem of the dearth of enough food supply and plant pathogens. Techniques such as slash-and-burn, pesticides and fertilisers are used for increasing yield as well as dealing with pests but do not contribute to agricultural sustainability (Harrison, 2002). Thus, current research needs to focus on developing cropping techniques which increase yield and mitigate the environmental impact (Foley et al., 2011). Nowadays, data-based computational tools are used to design agricultural strategies. Among others, the computational tools involve decision support models for choosing optimal cropping plans – which cultivar to grow and where – and crop rotation decisions (Castellazzi et al., 2008; Osman et al., 2015). The models guide allocating crops depending on their characteristics – botanical family, market demand, or soil demands –, examining the spatial distribution and temporal successions. However, these models need to integrate other farming concerns, one of which being the control of plant pathogens (Dun-chun et al., 2016).

In evolutionary biology, models on host-pathogen coevolution have contributed to understanding the relationship between some plant pathogens and their hosts in natural ecosystems, for example, regarding the specificity of the interaction (Agrawal and Lively, 2002; Flor, 1956). In domesticated crops, pathogen evolution is driven by natural selection in tandem with artificial selection: hosts do not

coevolve with the pathogen but, are instead, bred according to human interests. Recently, authors have highlighted the use of plant-pathogen evolutionary theory in formulating disease management strategies and avoiding the increase of infectivity in pathogens (Brown and Tellier, 2011; Burdon et al., 2014; Neve et al., 2009; Thrall et al., 2011; Zhan et al., 2014). Regarding evolutionary dynamics, theoretical approaches have studied trade-offs in plant-pathogen evolution linked to a periodic absence of host crops (Berg et al., 2011), a situation which resembles the phenomena in crop rotations. When rotating, a change in the crop type acts as a perturbation leading to frequent selective sweep-like dynamics. Tracking the frequency and speed of such sweeps would be useful in detecting periods of lower fitness and reduced population size; in which the pathogen could be pushed to extinction (Orr and Unckless, 2008). Then, adjusting the models used in natural plant-pathogen coevolution to the study of crop rotations can be a useful approach for tackling agricultural problems.

In this manuscript, we aim to design a cultivating strategy optimal for pathogen damage control, integrating agronomic criteria – soil quality and yield – used on crop rotations and pathogen evolution depending on the cultivated host. Our model for crop rotations focuses on patterns which maximise yield and appoint soil quality as a variable of interest. When infection occurs, ecological dynamics play out in the short term. Host-pathogen dynamics predicts crop loss depending on host susceptibility, as well as changes in pathogen load. We study pathogen evolution by including a transition of the pathogen into strains which can infect the host more efficiently. From all possible crop rotation patterns, only a few are good at maximising crop yield. Such patterns are shared among some tested pathosystems which have different characteristics. In general, an abundance of cover crop seasons is required as they play a double-role in both improving soil quality and breaking the epidemic. Knowledge of the initial soil status is shown to be vital in determining the actual best rotation. The field can only afford a 1:1 cash-cover crop ratio if nutrients are in excess from the beginning. The rotation patterns which maximise cash yield are assessed according to their performance over ten years, but also their ability to be used in a second and third decade. For sustainability over more extended periods, maintenance of soil quality and the minimisation of pathogen evolution into more infectious strains become central.

Overall, our computational model provides a generic framework which can be, at the interest of the researcher or farmer, adapted to particular plant-pathogen case studies. It provides guidelines, and it helps to understand the utility of crop rotations in pest management from an eco-evolutionary perspective.

## 3.4 MODEL AND METHODS

Plants have a variety of responses to pest infestations such as susceptibility, tolerance and resistance. In agriculture, farmers have used this variability for thousands of years to control the spread of pathogens. This simple yet powerful concept is formalised below, using a theoretical model which analyses the effect of rotation patterns in infection dynamics.

*Between-season model description: optimising rotations from soil quality*

To establish a basic model of rotation patterns, we focus on a sequential combination of cash crops and cover crops. Cash crops are those which provide a product to be commercialised – e.g. maize – whereas cover crops improve the soil quality of the field but provide no direct, substantial cash yield – e.g. clover. Since better soil quality provides more cash yield, including both crop types can result in improved farming: the basic model aims to study which temporal patterns of cash and cover crops maximise the farmer's benefit. We have been inspired by a previous report assessing the optimal length of clover period, compared to maize, in a 9-year field experiment (Boer et al., 2012). Here we work with pattern sequences of length $L$=10 seasons to acquaint long-term patterns with increased optimisation (Kierkegaard, 2003). We explore the space of possible cash-cover combinations of $L$=10 exhaustively and attribute each of the $2^L$ rotation sequences a yield value $Y$ which consists in the yield accumulated at the end of the ten seasons.

Each element in a rotation sequence corresponds to a harvesting season, modelled as a discrete time-step. During each time-step $t$, there is a change in soil quality $q(t)$ and cash yield $y(t)$, which varies depending on the crop type $i$=$\{1, 2\}$ (cash crops: $i$=1, cover crops: $i$=2)(Fig. 3.1).

The change in soil quality and cash yield, per time-step, are crucial in obtaining the final yield, taking the following form:

- Soil quality ($q(t)$): Soil quality decreases following a logistic decay curve for cash crops $c_1$ and increases with logistic growth for cover crops $c_2$. The parameter $\beta_i$ regulates the intensity and direction of the soil quality change given crop type $i$ at time $t$. We set $\beta_1 = -1.5$ for soil quality decrease by $c_1$, and $\beta_2$=1 for soil quality increase by $c_2$, considering that it is more difficult to improve soil quality than to decrease it (see SI for further examples). We assume that the soil quality cannot increase indefinitely, reaching a saturation value (carrying capacity) of $K$. We choose $K$=2, for which approximately $n_2$=4 harvesting seasons are needed to reach it with $\beta_2$=1, similar to the observations of a field report (Boer et al., 2012). In the logistic function, when

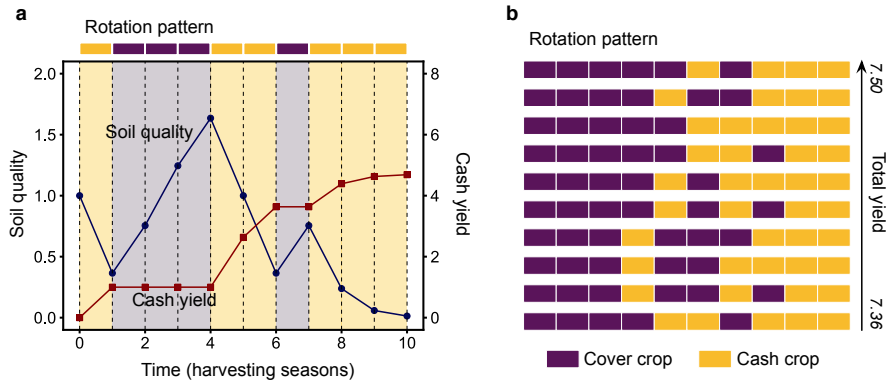


Figure 3.1: **Soil quality and cash yield variations in a rotation sequence and selection of 10 sequences that maximise cash yield.** a) Each time step corresponds to a harvesting season. Dots indicate discrete values for soil quality (blue circles) and cash yield (red squares). Season crop type is indicated by yellow (lighter) for cash crops and purple (darker) for cover crops. b) Ten optimal rotation patterns according to total cash yield $Y$. Each row is a rotation sequence, ordered from maximum to minimum yield (top to bottom) among the selection.

values are very close to the upper and bottom limits, the change is minimal both in growth and decay; hence we set thresholds of maximum $q(t)$=1.99 and minimum $q(t)$=0.01 from which soil quality does not vary, so changes become more perceptible. Also, the initial soil quality is set to $q(0)$=1, assuming the median value,

$$q(t+1)=max\left[0.01, min\left(1.99, \frac{Kq(t)e^{\beta_i}}{K+q(t)\left(e^{\beta_i}-1\right)}\right)\right]. \tag{3.1}$$

- Cumulative cash yield ($y(t)$): Cash yield increases in proportion to the soil quality at the beginning of the season $q(t)$, regulated by the crop yield contribution $\gamma_i$. For cash crops, we set $\gamma_1$=1, making cash yield increase in proportion to the soil quality, in a 1:1 ratio. For cover crops, there is no cash yield increase, $\gamma_2$=0 (see SI for further crop characterisation information). The cash yield accumulates along the rotation sequence time-steps,

$$y(t+1)=y(t)+\gamma_i q(t). \tag{3.2}$$

- Total yield ($Y$): This is simply the value of $y(t)$ evaluated at $t=L$. We define it separately since it is used as the main criteria to compare sequences and assess their optimality.

The time series of each possible rotation pattern in a population of sequences of length $L$=10 are computed according to the above-defined functions and analysed.

To understand the model predictions, we focus first on the top ten sequences whose patterns maximise cash yield and, hence, have a higher $Y$ (Fig. 3.1b). These

sequences share investment in – mostly – three consecutive cash crops during the last seasons, and they have the same number of cash and cover seasons. This information could be interesting for farmers to maximise their economic output, but it has two drawbacks: it does not take into account how the rotations perform under the real threat of pathogens and, not surprisingly, the soil quality by the end of the rotation is almost completely degraded. We assess each of these concerns in turn.

*Within-season dynamics: adding eco-evolutionary dynamics*

In natural settings, the process of coevolution between the host plant and its pathogen can lead to the cyclic evolution of host resistance and parasite virulence, maintaining genetic diversity (Brown and Tellier, 2011; Schenk et al., 2018). In agriculture, humans are the selecting agent: they decide which host grows in the next generation. While being economically significant, the selected crop can be particularly vulnerable to pathogens, which it has not been exposed to before. Moreover, there are only a few major agricultural cash crops; resulting in less genetic diversity in host crops and more disease susceptibility for the cultivars not selected for resistance (Anderson et al., 2004). In this section, we show how the introduction of a pathogen affects the assessment of a rotation sequences optimality. We start with a simple infection scenario in which a pathogen $p$ can infect cash crops $c_1$, but not the cover crops $c_2$, using the second as break crops (Angus et al., 2015). As an example, the fungi *Fusarium graminearum* is one such pathogen which infects cash crops like maize or wheat but does not infect cover crops such as clover (Marburger et al., 2015).

**Ecological dynamics.** To include host-pathogen ecological dynamics, we adapt the Lotka-Volterra competitive equations, based on (Song et al., 2015). Within a season, time is continuous, and dynamics described by a system of two ordinary differential equations,

$$\dot{c_i} = -c_i \sigma_i \sum_j p_j \tag{3.3}$$

$$\dot{p_j} = p_j \left( \sum_i \sigma_i c_i - d_j \right). \tag{3.4}$$

While the equations can allow for multiple hosts and pathogens, we start with two types of crop host ($i$=$\{1,2\}$) and a single pathogen ($j$=1). Here $c_i$ is the population density of crop $i$ and $p_j$ is the pathogen density. The pathogen infectivity is set by $\sigma_i$ ($\sigma_1$=0.04 for the susceptible cash crop, $\sigma_2$=0 for the cover resistant crop), and $d_j$ is the death rate of the pathogen ($d_j$=0.5). Due to the artificial setting of agriculture, we consider that without external perturbations and except for the pathogen-induced mortality, there is no birth nor death in the host population dur-

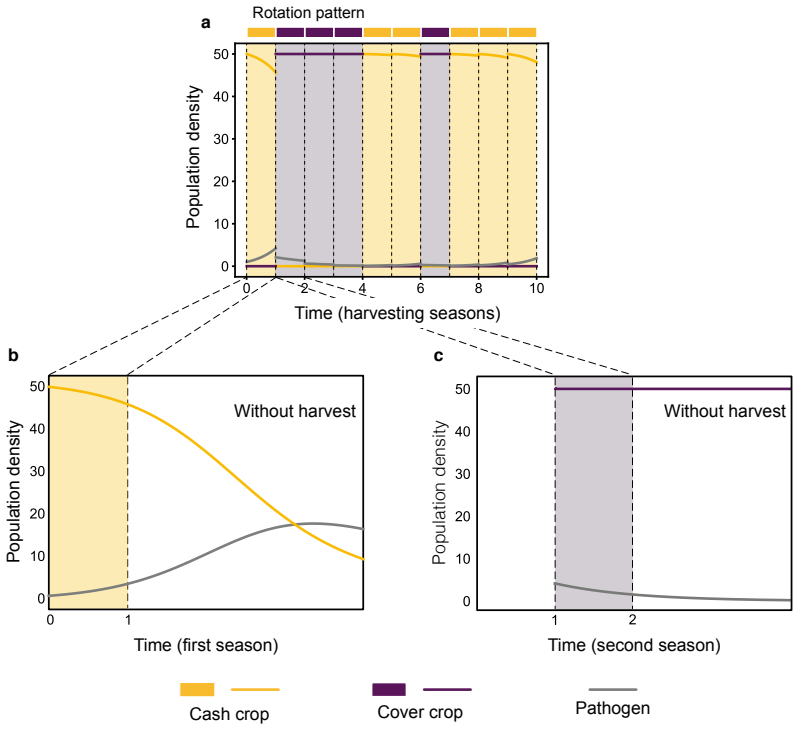


Figure 3.2: **Host-pathogen ecological dynamics, within and between seasons.** a) Dynamics between seasons. After each harvest, initial host density ($h0$=50) is reinitialised and pathogen density is readjusted according to the pathogen retainment ($\epsilon$=0.5). b) Dynamics within a season, when there is a susceptible cash crop ($i$=1). Host density decreases due to the presence of the pathogen, while the pathogen load increases as long as there are enough crops to infect. c) Dynamics within a season, when there is a non-host cover crop ($i$=2). The cover crop maintains its output while remaining unaffected by the pathogen. The pathogen dies since it cannot grow on the cover crop. Both b) and c) show how the dynamics would continue without the harvest.

ing the season. The host density declines when the pathogen is present according to its infectivity $\sigma_i$. This decline could be more complicated if we would include host tolerance with a pathogen density-dependent function regulating $\sigma_i$. In this study, we use a straightforward approach.

Change from one season to the next is a discrete-time step. At the end of each season, the crop is harvested, converted to yield, and new crops planted as per the rotation schedule. While harvesting, the pathogen population is disturbed. Some pathogens survive in soil or residues of the infected crops (pathogen retainment $\epsilon$). We set $\epsilon$=0.5, considering that half of the pathogen population stays in the field. Overall, the model evolves following continuous-time within seasons, and discrete jumps between seasons, being an example of a hybrid dynamical system (Schaft and Schumacher, 2000) as used for seasonal plant epidemiology (Madden and Bosch, 2002; Mailleret et al., 2012) (Fig. 3.2).

**Eco-evolutionary dynamics.** Substantial evolutionary changes can happen on ecological time-scales (Carroll et al., 2007; Frickel et al., 2016; Pelletier et al., 2009). Consequently, we need to include evolution in the host-pathogen interaction. In our case, we study the dynamics when there is the evolution of pathogen virulence, and incorporate them in the already developed ecological dynamics. In literature, virulence refers to the pathogen capacity to establish an infection or the consequences for the host to be infected (Gandon et al., 2002). We focus on the propensity of a pathogen to damage the host, through the regulation of $\sigma_i$. Within a season, the pathogen reproduces, generates variation, and some of these variants may carry mutations that provide more virulence. We do not include any costs for the additional infectivity. To incorporate evolution in the ecological dynamics, we modify the previous equations (Eqs. 3.3, 3.4) allowing the pathogen to mutate into strains which can exploit the cash host more efficiently (Eqs. 3.5, 3.6). The evolved pathogen cannot evolve to infect the cover crop since cash and cover are assumed to be phylogenetically distant and the cover is then a non-host species (Heath, 2003),

$$\dot{c_i} = -\sum_j W_{ji}\sigma_i c_i p_j \tag{3.5}$$

$$\dot{p_j} = \sum_k Q_{jk} p_k \sum_i W_{ki}\sigma_i c_i - p_j d_j. \tag{3.6}$$

The new equations have two critical elements: the transition matrix $Q_{kj}$ and the fitness matrix $W_{ji}$. The transition matrix $Q_{kj}$ corresponds to the rates in which the pathogen can mutate between five possible strains (Eq. 3.7). The strains are separated from each other by unit genetic distance. Thus to reach $p_5$ the original strain requires four mutational steps. Mutation can happen between strains which are one mutational step away with a transition rate $\mu$=0.1,

$$Q_{jk} = \begin{array}{c} \\ p_1 \\ p_2 \\ p_3 \\ p_4 \\ p_5 \end{array} \begin{array}{c} \begin{array}{ccccc} p_1 & p_2 & p_3 & p_4 & p_5 \end{array} \\ \begin{pmatrix} 1-\mu & \mu & 0 & 0 & 0 \\ \mu & 1-2\mu & \mu & 0 & 0 \\ 0 & \mu & 1-2\mu & \mu & 0 \\ 0 & 0 & \mu & 1-2\mu & \mu \\ 0 & 0 & 0 & \mu & 1-\mu \end{pmatrix} \end{array}. \tag{3.7}$$

For the fitness matrix $W_{ji}$, we set the fitness of the original pathogen $p_1$ to $w_{11}$=1 when infecting $c_1$; and to $w_{12}$=0 when infecting $c_2$. Each mutant increases the fitness proportional to the distance with respect to $p_1$, so $w_{j1}=w_{11}+0.25(j-1)$, with $w_{51}$=2 being the maximum fitness in our example system with five pathogen genotypes. Infecting $c_2$ does not provide any fitness benefit, with $w_{j2}$=0. The fitness matrix, when multiplied by the parameter $\sigma_i$, shows the increase in virulence in each mutated strain of the pathogen. In general, eco-evolutionary dynamics results in elevated crop loss as compared to the solely ecological dynamics (Fig. 3.3).

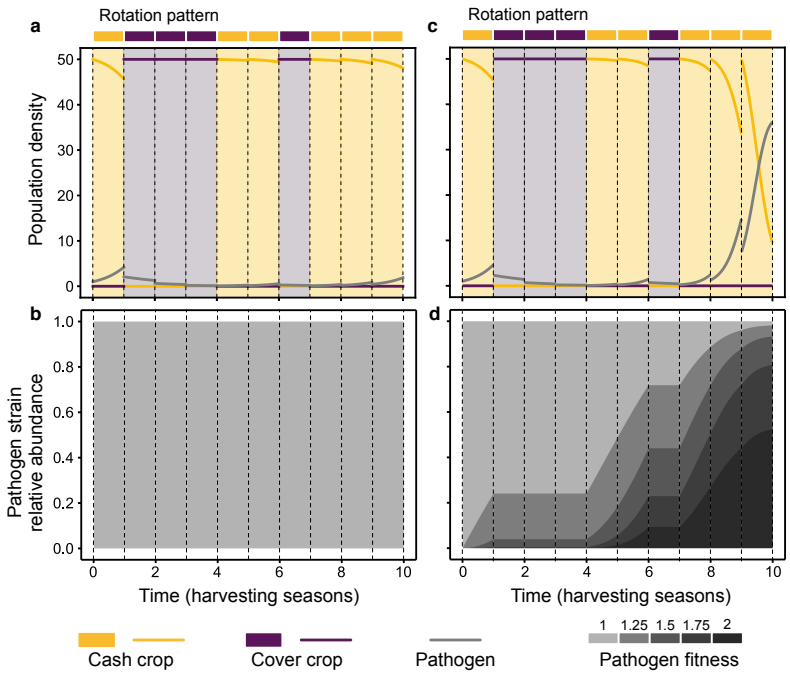


Figure 3.3: **Only ecological vs. eco-evolutionary dynamics of host-pathogen interaction.** a) Ecological dynamics, without pathogen evolution. Dynamics between seasons are represented, with infection starting at $t$=0 (as in Fig. 3.2a). b) The pathogen population is homogeneous, due to the absence of mutation. c) Eco-evolutionary dynamics with pathogen virulence evolution. Dynamics between seasons are represented, with infection starting at $t$=0. Due to pathogen evolution (with $\mu$=0.1), the impact of the infection in the last cash seasons provokes higher host density loss, compared to a). d) Time evolution of pathogen shows that the relative abundance of fitter strains – in darker colours – increases along seasons. In both b) and d), relative abundances of the pathogen strains are plotted.

*Coupling eco-evolutionary dynamics with yield loss*

When modelling the eco-evolutionary dynamics, pathogen growth decreases crop density. Therefore, those seasons which suffer infection do not have a cash yield outcome equivalent to their healthy counterparts. Consequently, the total yield $Y$ by which we choose the best sequences changes its value the more the crop is infected. To estimate the loss of crop yield, we modify the cash yield to consider the host density at the time of harvest: the effective crop ratio, or $\delta(t)$(Eq. 3.8). It indicates the proportion of the host population that is uninfected at the end of the season (dividing the crop density at the end of the season by the initial density),

$$\delta(t+1) = \frac{c_i(t+1)}{c_{i,t}} \tag{3.8}$$

$$y(t+1) = y(t)+\delta(t+1)\gamma_i q(t). \tag{3.9}$$

Table 3.1: List of fixed parameters used in the model

| Parameter | Description | Value | Reference |
|---|---|---|---|
| $\beta_i$ | Soil contribution of host $i$ | $\beta_1 = -1.5$, $\beta_2$=1 | |
| $\gamma_i$ | Cash contribution of host $i$ | $\gamma_1$=1, $\gamma_2$=0 | |
| $K$ | Carrying capacity of the soil | $K$=2 | |
| $\mu$ | Transition rate | $\mu$=0.1 | |
| $H$ | Initial host density | $H$=50 | |
| $\sigma_i$ | Infectivity of pathogen for host $i$ | $\sigma_1$=0.04,$\sigma_2$=0 | Song et al., 2015 |
| $d_p$ | Death rate of the pathogen $p_j$ | $d_j$=0.5 | Song et al., 2015 |

Included in the equation of cash yield (Eq. 3.9), $\delta(t)$ modifies the outcome of the season, so only uninfected crops contribute to the yield. Because even infected crops take nutrients from the soil, we do not include $\delta(t)$ in the soil quality equation.

All the parameters used in this modelling framework are collated in Table 3.1 and described with justification in the Supporting Information (see SI).

## 3.5 RESULTS

*Optimal rotation patterns under infection: the protective effect of cover crops*

Using the effective crop ratio $\delta(t)$ we compute the values of total yield $Y$ for each sequence. We model the scenario in which the pathogen infects at the beginning of the first season, at $t0$, and include pathogen evolution. As previously, we focus on the ten rotation patterns which yield the best (Fig. 3.4a). Interestingly, results show that 8 out of 10 rotation patterns which have a greater $Y$ in the presence of the pathogen coincide with the set of rotations that maximise yield in pathogen-free conditions.

Within the set of 10 optimal sequences, the yield range is $7.50 \leq Y \leq 7.36$ without infection and reduces to $7.49 \leq Y \leq 7.31$ with infection. In both sets, the best rotation pattern is the one starting with five seasons of cover crop, alternating after that and ending with three cash seasons. The reason behind the coincidence of patterns between the two sets is the double effect that the cover crops provide: on the one hand, they increase soil quality which in turn increases yield; on the

other hand, they break the epidemic diminishing crop loss and minimising yield loss.

*Sensitivity of optimal patterns to different pathogen and soil conditions*

Neither all epidemics have the same intensity, nor do all fields respond the same under the same farmer's practices. Here we explore the conditions under which our set of rotations can maximise yield and compare it with the sets of rotations which have a better outcome in other scenarios. By a set, we refer to the selection of 10 optimal sequences among the 1024 possible rotation patters. We also compare the maximum value of cash yield that we can get for each condition (Table 3.2).

**Pathogen retainment.** Crop rotations are used to control the disease, but not all pathogens are equally vulnerable to the effects of break crops, here cover crops. The spores of airborne pathogens, such as fungi, can disperse over long distances and are difficult to control with crop rotations because the infection often spreads from the neighbouring fields. Conversely, crop rotations can be beneficial for soil-borne pathogens which cannot reproduce on a non-host plant (Bullock, 1992; McGrath, 2009). The ability of pathogens to survive in the soil or in crop debris, which can also be modified by tillage practices, is represented in our model by the pathogen retainment ($\epsilon$). In the previous simulations, $\epsilon$=0.5, and here we explore what happens if its value increases to $\epsilon$=0.8 and decreases to $\epsilon$=0.2.

When we increase the retainment (set 1), the maximum yield decreases to $Y$=6.93, and the optimal sequences have a ratio of two cash crops for every three cover crops in all cases. The number of cover seasons increases because there is a need for a more extended period of non-host crops to compensate that more pathogen stays in the soil. When we decrease the retainment (set 2), the maximum yield is approximately maintained, being $Y$=7.50, and also the ratio of cash and cover crops.

**Initial pathogen inoculum.** For the pathogens, the characteristics of the initial inoculum can determine the severity of the epidemic (Jane-White and Gilligan, 2006). Here we explore it in two ways: the quantity of pathogen in the initial inoculum ($p_1(0)$) and the initial virulence of the pathogen, controlled by the values in the fitness matrix ($W_{ij}$) at time $t$=0. The default initial pathogen in our model is $p_1(0)$=1; here, we observe how a ten-fold increase $p_1(0)$=10 and decrease $p_1(0)$=0.1 affect the optimal rotation patterns and yield. For the pathogen fitness, we conserve the ability of the pathogen to mutate into five fitter strains, but we set values of $w_{11}$=1.5 and $w_{11}$=0.5 as initial fitness, in comparison to the reference $w_{11}$=1 (with $w_{j2}$=0, as before, for the cover crops $c_2$).

Starting with an initial pathogen of $p_1(0)=10$ (set 3) decreases the maximum yield to $Y=7.38$ and decreases the ratio of cash to cover crops to 2:3 in all the sequences. The decrease is not drastic since starting with five consecutive cover crops decreases the pathogen load. This feature is present also in the reference set, to increase soil quality, showing the double effect of the cover crops. The decrease of inoculum (set 4) maintains the yield to $Y=7.50$ and keeps the reference crop ratio. The increase of pathogen fitness (set 5) reduces the yield to $Y=6.91$ and decreases the cash to cover ratio to 2:3. The results of decreasing pathogen fitness (set 6) are similar to the decrease of initial inoculum, being the yield $Y=7.50$ and the ratio maintained to 1:1 or 2:3.

**Initial soil quality.** When farmers aim to maximise cash yield, disregarding soil quality can lead to a sterile field which needs more cover crops than *a priori* expected. Since the rotation plan may start in a field with poor quality, we check the effect of initial soil quality on the patterns. The values chosen are $q(0)=1.9$, close to the carrying capacity $K=2$, and $q(0)=0.1$.

High initial soil quality (set 7) leads to the highest maximum yield increase, being $Y=9.29$ and the ratio of crops 1:1. This yield increase is because we can get the highest yield in the first seasons, and we can maintain soil quality by the alternation of crops (Fig. 3.4b). The number of cash crops cannot increase more because this would promote the infection, decreasing the yield. Low initial soil quality (set 8) has the most substantial reduction of maximum yield, decreasing to $Y=5.30$. Dedicating more seasons in improving soil quality at the beginning, the ratio of cash to cover crops decays to 3:7 or 2:3 (Fig. 3.4b).

**Intersection of optimal sets.** Results show that the set of 10 best sequences shown in the previous section – and chosen as reference set – intersects with the optimal sets obtained in all conditions except for increased initial soil quality, despite changes in the maximum yield. We check for the number of common rotation sequences via a pairwise comparison of the sets for each of the exposed conditions (Fig. 3.4c).

The cases for which the sets intersect the most with the reference set relate to the initial pathogen: increase and decrease of pathogen retainment $\epsilon$ (8/10), increase (8/10) or decrease (9/10) of initial pathogen $p_1(0)$ and changes of initial pathogen fitness $w_{11}(0)$ (8/10). When pathogen retainment and pathogen fitness is high, there is full intersection due to a common need for more non-host crops that break the epidemic (Set 1 and Set 5, 10/10); and also vice-versa (Set 2 and Set 6, 10/10). Other conditions also have high intersection values between them (from 6/10 to 9/10) due to similar needs for both increasing soil quality and controlling

Table 3.2: **Yield and crop ratio for different pathogen and soil conditions**. Sets refer to the selection of 10 sequences which best maximise yield in each condition. Values in bold indicate the change of conditions in the set with respect to the reference set.

| Sequence set | Initial pathogen $p_1(0)$ | Pathogen retainment $\epsilon$ | Initial soil quality $q(0)$ | Initial pathogen fitness $w_{11}(0)$ | Maximum yield $Y_{max}$ | Cash: cover ratio |
|---|---|---|---|---|---|---|
| Ref. (R) | 1 | 0.5 | 1 | 1 | 7.49 | 2:3,1:1 |
| Set 1 | 1 | **0.8** | 1 | 1 | 6.93 | 2:3 |
| Set 2 | 1 | **0.2** | 1 | 1 | 7.50 | 1:1,2:3 |
| Set 3 | **10** | 0.5 | 1 | 1 | 7.38 | 2:3 |
| Set 4 | **0.1** | 0.5 | 1 | 1 | 7.50 | 2:3,1:1 |
| Set 5 | 1 | 0.5 | 1 | **1.5** | 6.91 | 2:3 |
| Set 6 | 1 | 0.5 | 1 | **0.5** | 7.50 | 1:1,2:3 |
| Set 7 | 1 | 0.5 | **1.9** | 1 | 9.29 | 1:1 |
| Set 8 | 1 | 0.5 | **0.1** | 1 | 5.30 | 3:7, 2:3 |

the infection. Variations in soil quality lead to the most different optimal patterns, with low (1/10) or no intersection with the rest of the sets.

*Longer-term rotations: soil quality and virulence control for the next generation of crops*

Ten seasons, or a decade in yearly crops, can be regarded as long term planning, but farmers cultivate fields for even longer. To investigate if our rotation patterns are sustainable over decades, we study the variation in the yield and the pathogen load in consecutively repeated patterns.

For the analysis, we focus on the repetition of the population of sequences of length $L$=10, i.e. all of the 1024 possible patterns and then repeating them twice or thrice. We term these repetitions as generations. We do not explore, however, the complete combinatorial space presented by the inclusion of second and third generation (i.e. $2^{20}$ or $2^{30}$ combinations), which is beyond the scope and focus of this manuscript. Of the 1024 patterns we limit our attention to the sets of 10 sequences that best maximise the yield in the infection scenario of reference ($p_1(0)$=1, $\epsilon$=0.5, $w_{11}(0)$=1) and median initial soil quality ($q(0)$=1).

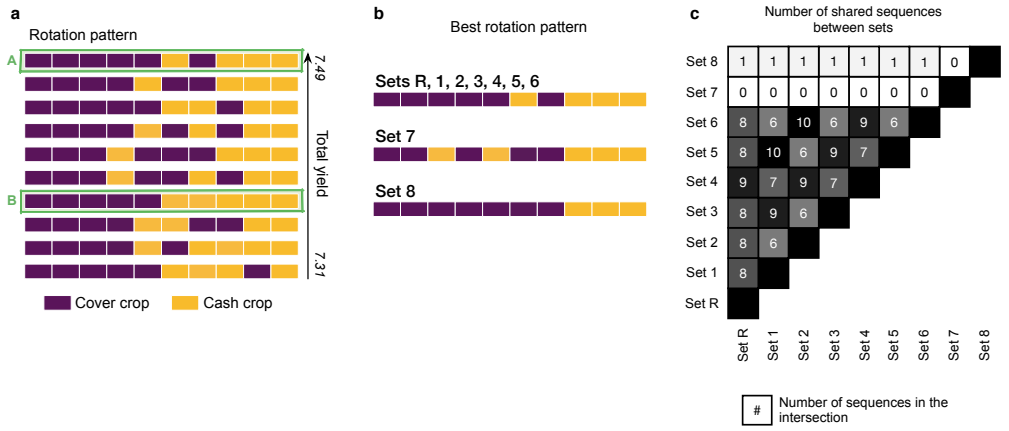


Figure 3.4: **Best patterns under infection in different conditions.** a) Selection of ten best patterns from 1024 possible sequences when cash yield loss due to infection is computed using the reference values. Each row is a rotation sequence. b) Best rotation sequence in the set of 10 optimal patterns for each of the conditions. The set index corresponds to conditions as indicated in Table 3.2. c) Intersection array for the sets of optimal sequences under different conditions. Each cell shows the number of sequences found in the intersection between the sets indicated in the vertical and horizontal labels. Highlighted sequences in (a): We allow for the 1024 possible sequences to repeat twice or thrice i.e. two or three generations. Rotation A is the sequence that maximises yield over multiple generations while Rotation B maximises yield only in the first generation but not later on.

The results show that the rotations that best maximise yield after the second and third generation coincide with the optimal rotations for the first generation (intersection of 8/10 for both sets). To further investigate their sustainability, we analyse the changes of the agronomic variables – soil quality and cash yield – and the host-pathogen eco-evolutionary dynamics. We focus on two rotation patterns: the common optimal rotation for all generations (Fig. 3.4, rotation A) and a rotation from the 10-optimal set of the first generation which is excluded in the set for the second and third generations (Fig. 3.4, rotation B). Rotation A starts with five cover crops, alternates for two seasons and finishes with three cash crops; rotation B has five cover crops followed by five cash crops.

The analysis (Table 3.3) shows that rotation A maintains the initial soil quality after the 10th season ($q(10)=1$), while rotation B depletes it ($q(10)=0.15$). In the previous section, we have shown that initial soil quality is key in determining the optimal rotation. Because of this feature, rotation A is able to maintain its optimal performance in the following generations, but B would need more investment in soil quality to aim for the same cash yield. Importantly, pathogen evolution during the first generation is also determinant in the yield outcome in the future. For rotation A, the increased frequency of virulent pathogen strains ($f(p_5)=0.28$) provokes more yield loss during the infection time. Consequently, the cash yield within the second ($Y=7.38$) and third ($Y=6.32$) generation is lower than within the first instance ($Y=7.49$), even if soil quality is maintained. This effect is more drastic for rotation B, which initiates the second generation with high frequency of virulent strains ($f(p_5)=0.46$) and shows severe infection when a cash crop is cultivated. The frequency of $p_5$ is chosen to be an indicator for virulence. If the strain $p_5$ exists then the existence of all other strains is guaranteed.

Remarkably, the pathogen strain with more fitness does not outcompete the rest of strains (Fig. 3.5). Since pathogens can mutate in both forward and reverse directions with the same rate (Eq. 3.5), the system reaches a mutation-selection balance in which the rate of generating strains with less fitness equals the rate at which the fitter strains are generated. The faster growth of the fitter strains is reflected in their higher eventual frequency in equilibrium.

These results show the properties of the rotation patterns that maintain soil quality and slow down pathogen evolution in the long term – requirements for sustainable farming.

Table 3.3: **Performance of rotation A and B along three generations.** A and B are the highlighted sequences in Fig. 3.4. Rotation A is the sequence that maximises yield over multiple generations. Rotation B maximises yield in the first generation but not in the subsequent. For each rotation and generation there are shown values for total yield, final soil quality and final frequency of the most virulent strain ($p_5$).

| | 1st generation | | | 2nd generation | | | 3rd generation | | |
|---|---|---|---|---|---|---|---|---|---|
| | Y | $q(10)$ | $f(p_5)(10)$ | Y | $q(20)$ | $f(p_5)(20)$ | Y | $q(30)$ | $f(p_5)(30)$ |
| A | 7.49 | 1 | 0.28 | 7.38 | 1 | 0.65 | 6.32 | 1 | 0.67 |
| B | 7.36 | 0.15 | 0.46 | 3.72 | 0.01 | 0.66 | 1.23 | 0.01 | 0.67 |

## 3.6 DISCUSSION

Translational evolutionary biology is a growing field where fundamental concepts from evolutionary biology can be used in an applied setting to make effective changes in society (Carroll et al., 2014; Fang and Casadevall, 2010). Just as with the search for novel antibiotics, the search for novel agricultural strategies can benefit immensely from evolutionary biology. Notably, applying evolutionary principles can help pest management in agroecosystems. Our work complements previous attempts on coupling plant genetics with resistance deployment strategies (Burdon et al., 2014; Zhan et al., 2015), but with a new focus on plant-pathogen dynamics and pathogen evolution in the context of crop rotation sequences.

We present a model for assessing how different patterns of cash and cover crop rotations influence long-term yield outcome and soil quality. Reported computational tools (Castellazzi et al., 2008; Dury et al., 2012; Osman et al., 2015) rely on historical data to predict which is the best decision. Typically farmers take a number of different factors (e.g. social, economical, biological and practical) into account when deciding upon an agricultural strategy. Our model indeed simplifies this complex decision-making process by choosing to focus on a smaller set of parameters such as soil quality, cash yield, infection dynamics and pathogen virulence evolution. In such a controlled setting, we are thus able to provide a *de novo* assessment integrating features of the crop field, epidemiology and pathogen evolution. Our results highlight that only a few patterns, from all possible crop rotation sequences, can maximise the yield. The resulting patterns suggest, broadly, investing in soil quality during the first seasons and once close to the carrying capacity of the soil, alternating the cultivation of cash and cover crops. By the end of the rotation, investment in cash crops maximises the yield of the decade.

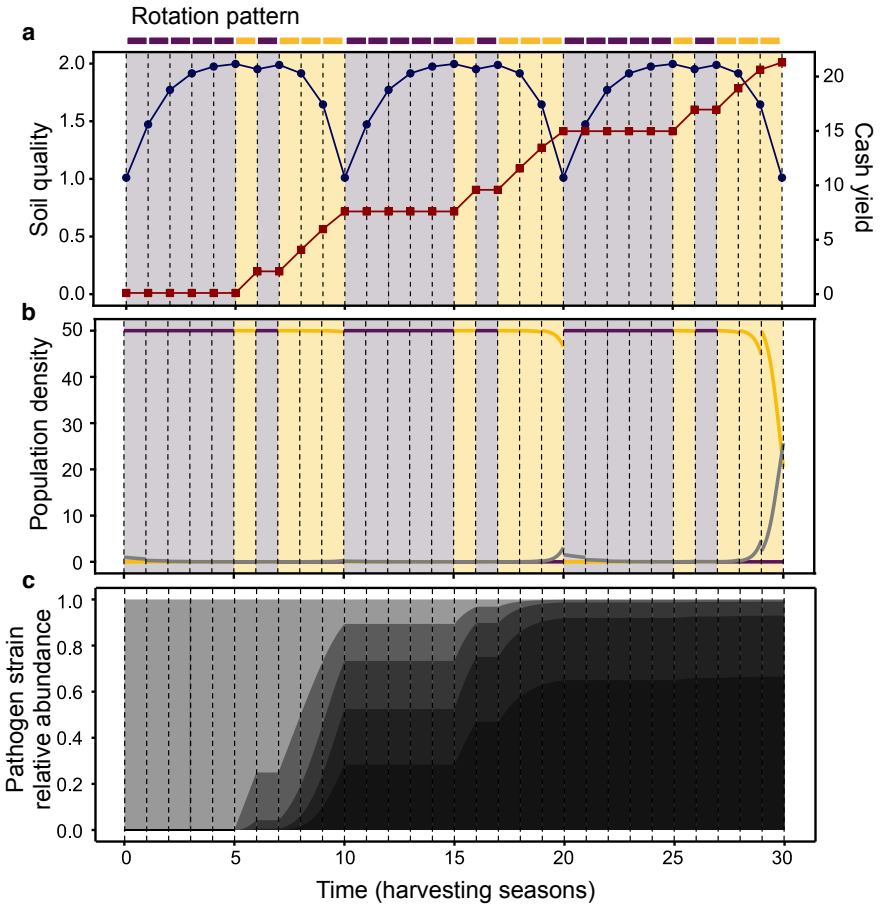


Figure 3.5: **Eco-evolutionary dynamics of rotation A when repeated thrice (30 seasons).** A) Soil quality (blue circles) and cash yield (red squares) variations, in discrete time-steps which correspond to the harvesting seasons. B) Eco-evolutionary dynamics of crop (yellow= cash, purple = cover) and pathogen (grey) within and between seasons. C) Relative abundances of pathogen strains during the rotation.

During the harvesting seasons, pathogens may invade the field and damage the crops, diminishing the expected yield. Using plant-pathogen dynamics, we have tracked the ecology and evolution of the infection in discrete and continuous time, predicting the possible magnitude of infection for each rotation sequence. By modelling pathogen ecology and evolution, we apply evolutionary biology concepts to agricultural strategies, as done in previous theoretical models (Burdon et al., 2014; Papaix et al., 2015), but we couple the dynamics with yield loss along with our rotations, to re-assess which rotations perform the best under infection. The resulting patterns coincide with the ones obtained under the no infection scenarios. The alternation of non-host cover crops with susceptible cash crops allows for efficient epidemic control and also to increase soil quality (and thus the yield), even in the absence of infection.

The rotations that maximise the yield depend on the conditions of the field and the epidemic. However, across several parameter values in our model, we observe consistency among the best patterns. These parameters are relevant to represent different plant pathosystems, as they characterise the initial pathogen, its retainment between seasons and its virulence for the host plant. The congruence in the rotation sequences could be significant for the farmers wishing to mitigate epidemics, when uncertain about the soil status or especially the presence of quiescent pathogens in the field. Based on a field history report, the initial conditions can be tuned to a specific rotation plan, thus adapting the model to desired crop characteristics for particular plant-pathogen case studies.

The patterns constrained by a limited time horizon always dedicate the final seasons to cash crops, depleting soil quality. However, in the long run, maintaining the levels of soil quality is necessary to have similar conditions after each rotation pattern, bringing the possibility of reapplying the sequence. The analysis of repetition of patterns for a second and third decade shows that investing more in cover crops is critical for long-term yield output. Acknowledging foresight, we are promoting the sustainability criterion (Howarth, 1995) in our system. The conditions presented for the first decade become similar to the ones that future decades will find so that the strategies can be maintained – perhaps with some variations due to external factors, e.g. climate.

From a sustainability point of view, besides soil quality, the capacity of the pathogen to evolve is also critical. In agriculture, most of the crop pathogens evolve rapidly, due to high planting density and genetic uniformity of the host, which increase the effective population size leading to more frequent random variation in the population (McDonald and Stukenbrock, 2016). Several well-known commercial varieties of crops suffer from such problems, such as the Cavendish bananas affected

by *Fusarium* wilt, also known as Panama disease (Ploetz, 2006). The strategies presented in this study do not eradicate the infection, but some rotations can delay the growth of more virulent strains. In the results, the sequences which yield the most in the second and third decade also have a slower increase of frequency of the virulent strains. The knowledge from our model could be coupled to current research that works on cultivar mixtures and crop mosaic patterning to diversify host genetics (Djidjou-Demasse et al., 2017; Mikaberidze et al., 2014). Our study emphasises the role of rotations in the long-term deployment of host resistance genes (Rimbaud et al., 2018), improving management practices for the delay of pathogen evolution.

While our model currently analyses a monoculture in a single field per season, it could be extended by including more variation in host types, spatial structure and between-field pathogen migration, complementing previous work (Fabre et al., 2015; Pacilly et al., 2018) with the crop rotations perspective. Also, an increase in the number of host types and the number of pathogens could lead to a model exhibiting complex, and even chaotic, dynamics (Schenk et al., 2017), which would be interesting to investigate. However, the most crucial step to take next would be the adaptation of the model to a particular case study.

As discussed during the analysis of optimal sequences under different conditions, not all pathogens respond the same to crop rotations, mainly depending on their life cycle. When focusing on microorganisms, soil-borne pathogens are commonly affected by the rotation practices, many of them being fungi. An example of crop disease with these conditions is the disease take-all of cereals (Hornby and Bateman, 1998), in which the fungi *Gaeumannomyces graminis var. tritici* causes root rot of the host plant, usually wheat or barley – cash crops in the model. For take-all, the use of a non-cereal crop as break crop – which would be in our model the cover crop – is useful for disease control. This particular crop-pathogen system is suitable for the theory as developed herein. Other crop diseases such as white mould, by *Sclerotinia sclerotiorum* (Adams and Ayers, 1979), would be a worthwhile investigation. The rotation scheme adapted to different crop types which all provide yield, as in the typical rotation of oilseed rape (host) and wheat (non-host) thereby will resulting in a modification of the theory to fit a specific model system.

Finally, we look at cash yield as the total economic benefit, without studying the costs of crop cultivation, and we do not emphasise how each sequence affects the farmers' seasonal benefit. Hence our model only loosely connects with the economics of agrosystems. Farmers' economic investment could be examined by including costs for each crop type. The short-term seasonal benefit can be regarded by simulating simultaneous rotation patterns for multiple subfields, with a minimum

number of cash crops per season, which would assure the yearly economic return and alleviate concerns over the discount-rate (Schelling, 1995). Additionally, the rational application of non-host crops can reduce the use of pesticides, and the associated balance of economic costs is a whole socio-economic project in itself which we aim to connect with evolutionary biology in the future.

## 3.7 CONCLUSION

Overall, our model can advise on strategies for maximising the gain of yield in cash crops, while using cover crops for soil improvement and control of pathogen spread. Further insights on rational resistance patterns could lead to new approaches for reducing pesticide use. Instead of applying the pesticide in all host seasons, the application could be limited to the host seasons where the pathogen density is low. This approach could both improve the efficiency of pesticides by increasing pest clearance and reducing the amount of pesticide used. Thus a synergistic use of crop rotations and pesticides can be possible together with biological control (Peterson et al., 2016). Experimental settings focus on crop rotations as the main factor for pest control, when put together with resistance variants and pesticides (Marburger et al., 2015). Agroecosystems rely on artificial selection for controlling the outcome of the harvest. We can profit from an evolutionary outlook bringing new tools towards more sustainable farming. Ideas such as the one presented in this research are essential first steps towards achieving this goal.

## 3.8 SUPPORTING INFORMATION

*Best sequences under different soil contribution ($\beta_i$)*

The parameter ($\beta_i$) captures how the planted crop affects soil quality. If cover crops improve soil quality rapidly and cash crops decrease it slowly, we would expect that optimal patterns have few cover crops, compared to the number of cash crops. In our case of study, we set $\beta_1 = -1.5$ for cash crops ($c_1$) and $\beta_2$=1 for cover crops ($c_2$), making soil quality decrease faster when one cash crop is cultivated, than the increase cover crops bring during one season.

Here, we analyse how the $\beta$ parameter space changes the ratio of cash and cover crops in the selection of rotation sequences which perform best on maximising cash yield in the pathogen-free scenario. We explore the cash:cover ratio for the combinations of values $\beta_1 \in \{0, -0.5, -1, -1.5, -2\}, \beta_2 \in \{0, 0.5, 1, 1.5, 2\}$. The mode values of the selection for each combination of $\beta$ is shown in a heatmap (Fig. 3.6).

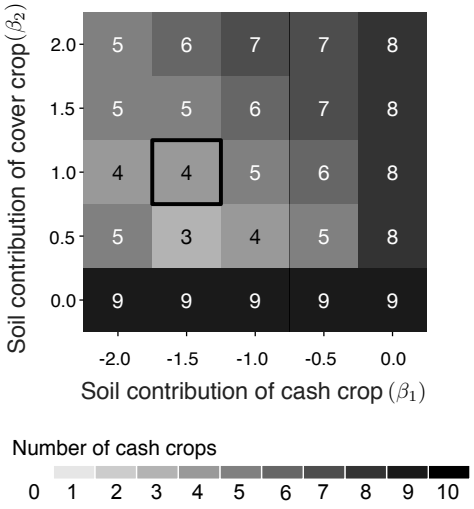


Figure 3.6: **Mode value of cash crops in the best rotation sequences for different values of soil contribution in cash and cover crops ($\beta_1$,$\beta_2$).** The heat map shows the mode value of cash crops in the selection of ten best rotation sequences for maximum yield in absence of infection. Each patch represents a combination of a value of $\beta_1 \in \{0, -0.5, -1, -1.5, -2\}$ and a value of $\beta_2 \in \{0, 0.5, 1, 1.5, 2\}$. The combination of values used for the results in the main text ($\beta_1 = -1.5, \beta_2 = 1$) is highlighted with a black square.

When $\beta_1$=0, for all $\beta_2$ values we get a high proportion of cash crops, compared to cover, in the selection of optimal sequences (number of cash crops $\geq$ 8). For $\beta_1 = -0.5$, the number of cash crops is equal or greater than the number of cover crops for all $\beta_2$ values (number of cash crops $\geq$ 5). If one season of cash or cover has the same (nonzero) magnitude of effect on the soil quality ($\beta_1$=$\beta_2$) then we get always a 1:1 ratio of crops. Other combinations vary in the ratio, being the lowest a number of 3 cash crops when $\beta_1 = -1.5$ and $\beta_2$=0.5. For $\beta_2$=0 without infection, the best strategy is always to cultivate cash crops: even if we deplete the soil quality, the cash gain is greater than 0 (being the minimum soil quality $q(t)$=0.01). The ten best sequences would then consist of one all cash sequence and nine sequences with one cover crop at different seasons. Thus overall, all combinations with $\beta_2$=0 show a mode value of 9 cash crops.

*Characterisation of different types of crops*

In the main article, we have used two types of crop, designed as cover and cash crops and given specific parameter values. However, we can characterise a diversity of crops by using the parameters of soil contribution ($\beta_i$) and cash contribution ($\gamma_i$). Typically cover crops are defined as the crops that help increase the soil quality (positive $\beta_i$). These cover crops are often not directly related to the crop budget or

do not have a direct payoff – in our model – low values of cash contribution ($\gamma_i$).

Cash crops are characterised by resulting high cash contribution ($\gamma_i$). However, cash crops usually deplete the soil of nutrients (negative values of soil contribution $\beta_i$) – creating a trade-off between the parameters. Picturing the crops in a space defined by the two parameters $\beta_i$ and $\gamma_i$, those regions with high positive values for both would appear empty due to these trade-offs, and those regions with negative values of $\beta_i$ and null $\gamma_i$ would not belong to crops of interest.

Finally, not only the soil contribution ($\beta_i$) and cash contribution ($\gamma_i$) characterise crops, but also the infectivity $\sigma_i$ that the pathogen has for them. This dependence provides the opportunity to study further crop combinations with, for example, cash variants which have resistance to the pathogen – lowering $\sigma_i$ – and pay a yield cost for such resistance – decrease in $\gamma_i$. Similarly, the pathogen could have different fitness values for both the cash crop and the cover crop – which could be replaced by a host crop with partial resistance ($w_{j2} > 0$).

*Fixed parameters*

In the model, there are several parameters which have fixed values. We have compiled them in Table 3.1, shown in the main text. Besides the infectivity and the death rate of the pathogen, which have the same values as in previous models, we have set the values for the rest. The soil ($\beta_i$) and cash ($\gamma_i$) contribution of the cash ($c_1$) and cover ($c_2$) crops have values according to the qualitative effect of their type of crop. During the same period, a cash crop decreases soil quality faster than a cover crop can recover it; and cover crops do not provide cash yield. The soil quality can be improved only up to a carrying capacity of $K$. The relative values of $K$ and $\beta_2$ are chosen so as to reflect field observations (after four seasons of cover crops, the change in soil quality is not noticeable (Boer et al., 2012). The pathogen strain transition rate $\mu$ has a high value due to the quick adaptation of pathogens to hosts in agro-ecosystems (McDonald and Stukenbrock, 2016). The initial host density has an arbitrary value, but this does not affect the optimality of the sequences because the crop loss is proportional to the initial host density.

# 4

# ASSESSING INTEGRATED MANAGEMENT PRACTICES FOR CONTROL OF *SCLEROTINIA SCLEROTIORUM* IN OILSEED RAPE

The content of this chapter is part of an on-going manuscript, as a collaboration with Alice Milne, Nichola Hawkins and Joe Helps from Rothamsted Research, and Chaitanya S. Gokhale from the Max Planck Institute for Evolutionary Biology.

The study is designed as an application of the generic model of host-pathogen dynamics in crop rotations presented in Chapter 3. The adaptation of the generic model to a case study requires a more detailed evaluation of the biology of the host crop and the pathogen, leading to modifications and space for improvement. In this case, I work with white mould of oilseed rape because of being a soil-borne pathogen which farmers control using crop rotations, among other techniques. Also, oilseed rape is a crop of commercial interest for many countries, being the second major oilcrop most produced in the world, after soybean (FAO, 2018).

The `Python` core codes, describing the model, are available on `Github` at *https://github.com/tecoevo/sclerotinia*.

## 4.1 ABSTRACT

Sclerotinia stem rot, or white mould, is a plant disease caused by the fungus *Sclerotinia sclerotiorum*. It affects a wide range of crops, such as oilseed rape, lettuce, carrots, sunflower and soybean. Here, we focus on oilseed rape as the host plant and study the development of Sclerotinia when integrated pest management is applied to control the disease. We analyse the effects of cultural control – i.e. rotations with break crops –, application of fungicides, and the use of host genetic resistance; both separately and combined. For that, we use a model with hybrid time dynamics: within a season, the infection follows disease spread in continuous time; between seasons, time is discrete, and host and pathogen population densities are updated. We simulate consecutive seasons of a single field, where we track the number of fungi that stays in the soil and the yield gain at the end of the harvest. The goal is to study the control measures that prevent a build-up of the infection and minimise the yield loss in the long run. Results show that the integration of strategies is successful in controlling the pest even when rotations are short (1-year break between oilseed rape seasons). Further assessment of integrated management

practices requires an analysis of the cost-benefit balance of each strategy, and the study of pathogen evolution to determine the durability of host genetic resistance and fungicide control methods.

## 4.2 INTRODUCTION

Every year, plant diseases affect crops, diminishing the farmers' yields. Sclerotinia stem rot (SSR) is a disease caused by the fungi *Sclerotinia sclerotiorum* which affects several commercial crops causing white mould and stem rot. Here we focus on how it affects oilseed rape (OSR), *Brassica napus*, where infection can cause yield losses of up to 60% (Rothmann and McLaren, 2018; Twengstrom et al., 1998). The epidemic can be reduced by using crop rotations or foliar fungicides, but farmers rarely achieve total control using a single method. Integrated pest management (IPM) is regarded as a potential solution (Derbyshire and Denton-Giles, 2016). IPM, by definition, takes genetic, chemical and biological control as complementary, and uses all suitable techniques to maintain the population levels of a pathogen below those causing economic losses (Kogan, 1998; Smith and Reynolds, 1966). The use of IPM in SSR could improve current practices by combining both crop rotations and fungicides, and adding alternative strategies such as crop variants with partial genetic resistance or biocontrol (Derbyshire and Denton-Giles, 2016). However, the idea needs further exploration from both theoretical and experimental sides. The characterisation of the synergistic effect of integrated practices is required previous to field applications, as the use of multiple strategies has an increased economic cost for the grower, who needs compensation in the outcome.

*Sclerotinia sclerotiorum* is a soil-borne fungus which proliferates in moist conditions and has a monocyclic life cycle, i.e. it relies on a primary inoculum to develop, without producing secondary inocula. To survive, it forms a structure called sclerotia which allows it to remain in the soil across seasons. When the sclerotium germinates, it produces fruiting bodies which release ascospores to infect the host. In OSR, the ascospores infect the petals in the flowering season, which fall and cause damage to the stem. In the infected stems, more sclerotia are produced, reaching the soil and closing the life cycle (Derbyshire and Denton-Giles, 2016; Heffer and Johnson, 2007) (Fig. 4.1). The severity of the disease depends very much on weather conditions – it is favoured by high humidity and warm temperatures –, but farming practices are also considered when assessing the risk, mainly the OSR cropping frequency in the field (Koch et al., 2007).

A common cultural practice that prevents SSR is the rotation with cereals such as wheat or barley. Because these cereals are non-hosts for the pathogen, the remnants of sclerotia in the soil are reduced during the season (Gracia-Garza et al.,

2002). However, sclerotia can remain viable in the soil for up to 8–10 years (Adams and Ayers, 1979), requiring farmers to apply long rotations with multiple years of break crops. Otherwise, in short rotations, the infection can build up from season to season due to the accumulation of sclerotia: this phenomenon has accentuated in the last decade as the frequency of OSR in rotations has increased from more than four break seasons to 1-year break (Berry et al., 2014). The use of fungicides offers another viable control method. The application of fungicides in the early flowering time as prophylaxis can prevent the infection. However, if the ascospores have reached the petals before the spraying or the risk of infection is very low, the fungicides have limited effect and so this results in economic losses (Koch et al., 2007). There are no known cultivars with full resistance to SSR, but varieties with partial resistance or some disease tolerance have been identified (Garg et al., 2010; Taylor et al., 2015).

Here, we study IPM strategies to control SSR of OSR by adapting a generic model of seasonal rotations of host and non-host crops (Bargués-Ribera and Gokhale, 2020). Taking the implementation of seasonal rotations, we expand the model to include both the fungicide effect and partial resistant crop variants. We aim to study how the integration of two or three control strategies – rotations, fungicides and genetic resistance – can improve the management of OSR. For that, we simulate host infection dynamics within and between seasons. The season output is coupled to two variables of interest: yield gain and spore bank, i.e. changes of sclerotia quantities in the soil. Overall, the model informs that combined strategies can succeed in controlling the infection using short rotations, even if the fungicide efficiency and the level of genetic resistance are low.

## 4.3 MODEL AND METHODS

### *Model description*

The model is based on Bargués-Ribera and Gokhale (2020), which focuses on crop rotations in a single field. In each season, one crop type is cultivated: a host crop promotes the infection, while a non-host crop prevents it. Plant-pathogen dynamics are modelled within and across harvesting seasons. The model uses hybrid time: within a season, the disease follows infection spread in continuous time; between seasons, time is discrete and population densities are updated. The infection is coupled to the calculation of seasonal yield.

In the model presented here, the host crop is oilseed rape (OSR). Disease control can be achieved by rotations with break crops (non-host) as a form of cultural control (C); but in contrast to the previous model, we extend management strategies to the

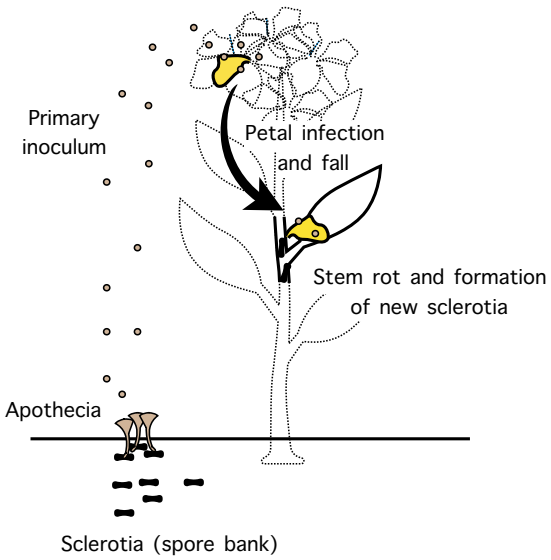


Figure 4.1: **Life cycle of *Sclerotinia sclerotiorum* in oilseed rape.** During the flowering season of the crop, the sclerotia structures in the soil produce germinating apothecia, which release ascospores. The ascospores are the primary inoculum that infects the petals of oilseed rape flowers. When the petals fall, they infect other plant areas, provoking stem rot. In the infected stem, new sclerotia are formed. Sclerotia reach back the soil when the stem breaks and lodges.

use of genetic resistance (G) and the application of fungicides (F). Infection is modelled from the first season. In each season, we model healthy and infected crop densities. After each season, two discrete variables are updated according to the status of the infection: spore bank (equivalent to the number of soil sclerotia) and yield. We explore infection dynamics in rotation sequences of 20 seasons of length, and we assess the total OSR yield and infection build-up based on the final spore bank. Results are compared for the cultivation of consecutive OSR (null case) and the use of one, two or three control strategies (C, G, F) (Fig. 4.2).

*Within and between season infection*

The infection is modelled using a system of two coupled differential equations which indicate the dynamics of the healthy and the infected crop density. Initially, there is a total crop density of 100 plants/m$^2$, considering both healthy and infected densities ($h(t{=}0){+}i(t{=}0){=}100$). Healthy plants do not reproduce, their density decreases when they get infected at rate $\beta$ (Eq. 4.1). The primary inoculum ($i_0$) determines the initial value of the infected crop density ($i(t{=}0)$), which value increases during the season. This increase depends on the rate of infection $\beta$, the available healthy crop density $h(t)$, the current infected density $i(t)$ and the influx of primary inoculum $i_0$ (Eq. 4.2). The infected plants die at a rate of $\delta$.

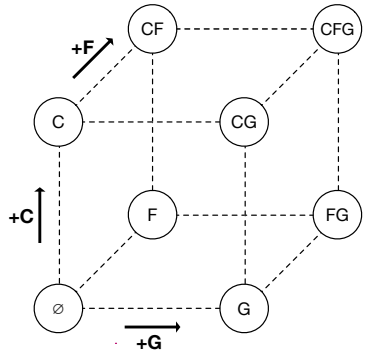


Figure 4.2: **Space of possible control strategies.** Starting from the null strategy (∅), we can apply either cultural (C), fungicide (F) or genetic (G) control. From these points, we can add a second strategy and use cultural-fungicide (CF), cultural-genetic (CG) or fungicide-genetic (FG) double control methods. Finally, we can combine the three strategies and use cultural-fungicide-genetic control (CFG).

At the end of the season, we calculate the area under disease progress curve AUDPC ($A$), which indicates disease intensity over time. To do so, we approximate the area under the curve (i.e. the definite integral) of the infected plant density. We use the trapezoidal method (Madden et al., 2007) using the beginning and the end of the season as time interval (Eq. 4.7). With the AUDPC we update the spore bank $S(t)$, which conserves a proportion $\eta$ from the last season and adds sclerotia during the infection of the current season $\gamma A$ (Eq. 4.4). The yield for each season ($Y(t)$) depends on the crop infection status at the moment of the harvest, i.e. the final value of $i(t)$ (Eq. 4.5). The primary inoculum for the next season is set according to the spore bank using $\epsilon$. If fungicides are applied, the primary inoculum is reduced by 1-$\mu$, where $\mu$ is the fungicide efficiency (Eq. 4.6). The spore bank builds up when there are consecutive seasons of the susceptible crop. If a break crop is grown then there is no build up in the spore bank and so the infection potential is reduced. With a resistant crop, the pathogen fitness $w$ is reduced depending on $\rho$, which lowers the rate of infection spread and so the increase in spore bank is smaller.

$$\dot{h} = -w\beta h(i+i_0) \tag{4.1}$$

$$\dot{i} = w\beta h(i+i_0) - \delta i \tag{4.2}$$

$$w = 1-\rho \tag{4.3}$$

$$S(t) = \eta S(t-1)+\gamma A(t) \tag{4.4}$$

$$Y(t) = Y_{max} - \alpha i(t) \tag{4.5}$$

$$i_0 = (1-\mu)\epsilon S(t) \tag{4.6}$$

$$A(t) = \frac{i(t-1)+i(t)}{2}\Delta t \tag{4.7}$$

*Characterisation of cropping strategies*

The dynamics within one season depend on the initial inoculum $i_0$ and the rate of infection spread $\beta$; which lead to an end-of-season stem rot severity $i(t)$. The initial inoculum calculates on the amount of spore bank $S(t)$, and it changes with the application of fungicides, with fungicide efficiency $\mu$. The rate of infection spread varies with the crop type: when the crop has some resistance, pathogen fitness is decreased ($w < 1$). The four management interventions that are explored are:

- No control (null strategy, $\emptyset$). We grow consecutive seasons of a susceptible variety of oilseed rape (all OSR). Because the crop is susceptible, the pathogen has full fitness for the host crop ($w$=1). Fungicides are not applied ($\mu$=0).
- Cultural control (single strategy, C). We alternate oilseed rape and break crops in seasonal rotations (repetition of OSR-BC). In seasons of OSR, the pathogen has full fitness for the host crop ($w$=1). In seasons of break crop, the primary inoculum is null ($i_0$=0). Fungicides are not applied ($\mu$=0).
- Fungicide control (single strategy, F). We cultivate consecutive seasons of oilseed rape (all OSR). Because the crop is susceptible, the pathogen has full fitness for the host crop ($w$=1). Fungicides are applied ($\mu > 0$), reducing the primary inoculum.
- Genetic control (single strategy, G). We grow consecutive seasons of oilseed rape (all OSR). However, the pathogen is assumed to have a reduced ability to infect this crop compared to the susceptible. This is simulated by reducing the pathogen fitness for the host crop ($w < 1$). Fungicides are not applied ($\mu$=0).

When the control strategies are applied together, the specifications of each strategy are combined, as shown in Table 4.1.

*Parametrisation*

The equations have several parameters (Table 4.2) which values define the infection dynamics. In consequence, their optimisation can approximate the results observed in crop fields. Here we describe the relationship of these parameters with descriptive variables found in field reports or previous literature. We show an example table (Table 4.3) from Gladders et al. (2008), which shows values for stem rot index and yield for untreated and treated fields of winter OSR in the years 2006 and 2007, when there was a severe epidemic of SSR in the United Kingdom. From all values, we have selected the fields Hereford 1 and 3, the year 2007, as their cultivar varieties (Catalina and Castille, respectively) showed both severe stem rot

Table 4.1: **List of strategies and their characterisation.** There are eight strategies: null(∅), single cultural (C), genetic (G) and fungicide (F) control, and the combinations of single control methods. Each of them is characterised according to the seasonal pattern (consecutive OSR, or rotations with break crop OSR-BC), the pathogen fitness ($w$) and the fungicide efficiency ($\mu$).

| Strategy | Seasonal pattern | Pathogen fitness | Fungicide efficiency |
|---|---|---|---|
| ∅ | OSR | $w$=1 | $\mu$=0 |
| C | OSR-BC | $w$=1 | $\mu$=0 |
| G | OSR | $w < 1$ | $\mu$=0 |
| F | OSR | $w$=1 | $\mu > 0$ |
| CG | OSR-BC | $w < 1$ | $\mu$=0 |
| CF | OSR-BC | $w$=1 | $\mu > 0$ |
| GF | OSR | $w < 1$ | $\mu > 0$ |
| CGF | OSR-BC | $w < 1$ | $\mu > 0$ |

and a significant response to fungicide treatment. We can use it as a guide for parametrisation, as explained:

- Within a season, the **rate of infection** $\beta$ determines the infected plant density at the end of the season $i$. In the report, we can relate $i$ to the **stem rot index**. The stem rot index determines the percentage of infected plants, and it is used as indication of disease severity. In the data, the average stem rot index, without treatment, is 60.5% (s = 10.6). In our simulation, when starting with an initial spore bank of $S(t{=}0){=}50$ – which could relate to severe infection – $i(t{=}1){=}66.36$. Compared to the total plant density ($i(t{=}1){+}h(t{=}1){=}89.67$), this represents a 74% of infected plants. Thus, infection is overestimated according to the assumed initial inoculum. These results also depend on the **death rate of infected plants** $\delta$, for which we do not have any data.

- Within season dynamics also determine the **yield**. In the report, the infected fields yielded on average 2.6 t/ha. The maximum yield with treatment was 4.7 t/ha. This can be indicative of an approximated maximum yield of 5 t/ha without infection. An $Y_{max} = 5$t/ha is realistic for winter oilseed rape cultivars with high yield (Storer et al., 2018) and corresponds to approximatively 50% of yield loss in the data for severe infection. When calculating yield under infection, we use a function of the infected area which determines **yield loss** according to $\alpha$. Oilseed rape yield depends on the plant seeds, which are reduced or damaged when the plant dies or the infection is severe. Usually yield loss does not exceed 50-60 %. In our simulations, for values in Table

Table 4.2: **List of parameter values.** The parameters presented define the infection according to Eqs. 4.1-7. The description indicates their role in the infection. They are assigned arbitrary values to test the model, within the corresponding range.

| Parameter | Value | Description | Range |
|---|---|---|---|
| $\alpha$ | 0.035 | Yield loss due to infection | 0 - 0.05 |
| $\beta$ | 0.04 | Rate of infection | 0 - 1 |
| $\gamma$ | 1 | Seasonal spore gain | 0 - 1 |
| $\delta$ | 0.3 | Death rate of infected plants | 0 - 1 |
| $\epsilon$ | 0.15 | Seasonal inoculum | 0 - 1 |
| $\eta$ | 0.6 | Seasonal spore survival | 0 - 1 |
| $\mu$ | variable | Fungicide efficiency | 0 - 1 |
| $\rho$ | variable | Genetic resistance | 0 - 1 |

4.2 and initial spore bank of $S(t{=}0){=}50$, we obtain a yield of 2.7 t/ha, close to the data average, and corresponding to a 46% of yield loss.

- Between seasons, a proportion of the spore bank remains in the soil. The half life of sclerotia in cultivated soil layers is estimated to be 2.5 years (Archer et al., 1992), but it can survive up to 8-10 years (Adams and Ayers, 1979). The parameter $\eta$ regulates the **decay of soil sclerotia**. In our simulations, the spore bank is reduced to 50% after approximatively 2.2 seasons of break crop (null increase). After 5 seasons, it is reduced to less than 10 % of the initial value. After 10 seasons, less than 1% of the initial spore bank remains (Fig. 4.3).

- Regarding fungicide application, the table compares stem rot index in untreated and treated fields, which can approximate the efficiency of the fungicide. However, in our study we focus on the exploration of the **fungicide efficiency parameter** $\mu$, as the resulting control can depend on the fungicide type and dosage.

The parameter values can be fit to the field report or other sets of data, using optimisation algorithms that adjust them to obtain the expected results (see discussion). The current values aim to be useful to test the model and explore general outcomes of the integration of strategies.

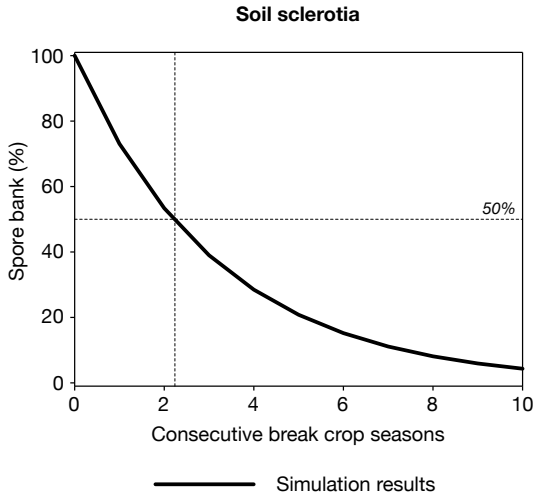


Figure 4.3: **Decay of soil sclerotia with consecutive seasons of break crops.** The parameter $\eta$ regulates the proportion of sclerotia that remain in the soil from season to season (here, $\eta$=0.6). When break crops are cultivated, no new sclerotia are produced and soil sclerotia decays (black line). With the current parametrisation, after 3 consecutive break crop seasons the number of soil sclerotia is reduced to more than a half (dotted line indicates intersection between break crop seasons and half the spore bank).

Table 4.3: **Field experiments data for SSR in winter oilseed rape.*Adapted from Gladders et al. (2008).*** Stem rot severity pre-harvest, untreated yield and yield response to a flowering fungicide application in individual field experiments in England, 2007.

| Site | Year | Cultivar | Stem rot index (untreated) | Stem rot index (treated) | Untreated yield | Treated yield |
|---|---|---|---|---|---|---|
| Hereford 1 | 2007 | Catalina | 57.3 | 9.6 | 3.0 | 4.7 |
| Hereford 3 | 2007 | Castille | 72.3 | 13.0 | 2.6 | 3.4 |
| Hereford 1 | 2007 | Catalina | 67.7 | 11.7 | 2.4 | 4.5 |
| Hereford 3 | 2007 | Castille | 44.8 | 8.4 | 2.4 | 3.3 |
| | | Average | 60.5 (s = 10.6) | 10.7 (s = 1.8) | 2.6 (s = 0.2) | 4.0 (s = 0.6) |
| | | Defined range | 0-100 | 0-100 | 0-5 | 0-5 |

## 4.4 RESULTS

The results show the performance for null (∅), single (C,F,G), double (CF, CG, FG) and triple (CFG) strategies during 20 seasons, in terms of yield gain and build-up of sclerotia in soil. These results are constrained to an infection which develops from an initial spore bank of $S(t{=}0){=}10$, unless specified. Also, we do not consider pathogen evolution, i.e. we assume durable effectiveness of genetic resistance and fungicide application.

*Null strategy (∅) and cultural control strategy (C)*

**Null strategy (∅)**. We simulate 20 consecutive seasons of OSR. At the end of the 20th season, the values for infected host density and spore bank are maximum in respect to other seasons ($i(t{=}20){=}73.76$ plants/m$^2$, $S(t{=}20){=}113.36$ a.u.) (Fig. 4.4). The infection dynamics show a rapid build-up of soil sclerotia in the initial seasons: considering the final spore bank as 100% of build-up, spore bank at the end of the 6th season reaches more than 80% of build-up ($S(t{=}6){=}94.77$ a.u.). The yield obtained per season decays with time, and at the end of the 20th season we get 48.40% of the maximum yield ($Y(t{=}20){=}2.42$ t/ha). When comparing with the cumulative maximum yield of OSR – i.e. total yield collected after 20 seasons with 5 t/ha each –, we get 51.12 % of the total.

**Single strategy: cultural control (C)**. We simulate 20 seasons of the rotation OSR-BC (i.e. seasonal alternations of OSR with a break crop BC). We establish the rotation with a single season of break crop (short rotation) as the default cultural strategy, but we explore how the results change when we increase the number of break crops (long rotation) (Fig. 4.4). The infection dynamics show that short rotations (OSR-BC) do not prevent a build-up of the infection: the spore bank after the 20th season is increased respect to the initial one ($S(t{=}20){=}29.02$ a.u., $S(t{=}20) > S(t{=}0)$). However, longer rotations with three seasons of break crop (OSR-BC-BC-BC) do control the infection and reduce the sclerotia in soil ($S(t{=}20){=}1.19$ a.u., $S(t{=}20) < S(t{=}0)$). The maximum infected host density occurs at the last season of OSR for short rotations ($i(t{=}20){=}57.73$ plants/m$^2$) and the first season of OSR for 3-break crop rotations ($i(t{=}1){=}35.83$ plants/m$^2$).

When applying cultural control, the cumulative maximum yield of OSR corresponds to the total yield collected during the OSR seasons: 10 seasons in the case of short rotations, 5 seasons for long rotations with 3 break crops. Taking this into account, we get 63.32% of the total yield for short rotations and 86.48% of the total yield for long rotations with 3 break crops.

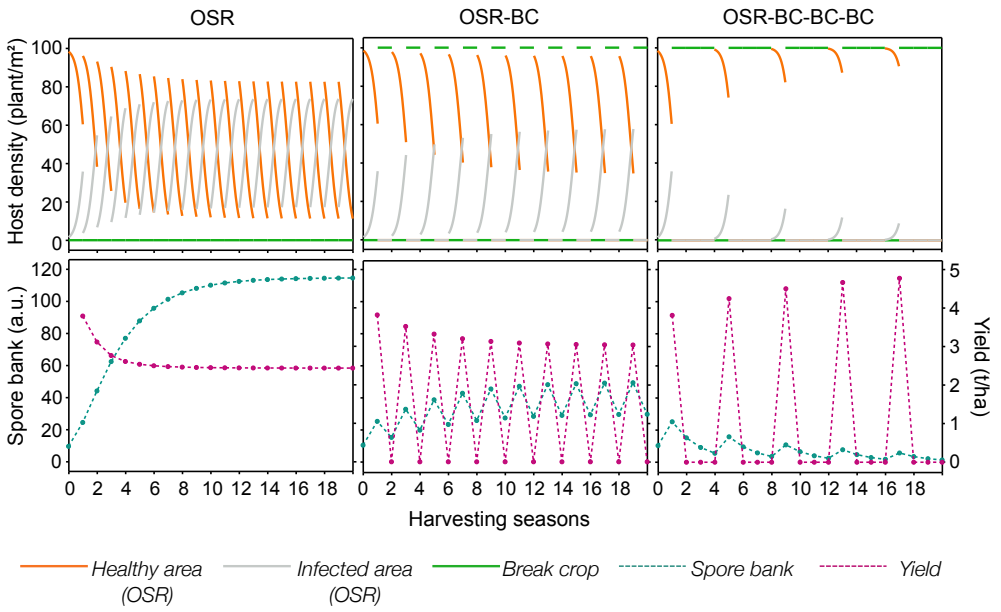


Figure 4.4: **Infection dynamics, with spore bank and yield seasonal updates, for the null (∅) and cultural control (C) strategies, during 20 harvesting seasons.** Top panels indicate infection dynamics, which show variations in healthy crop area (orange for oilseed rape, green for break crop) and infected crop area (grey). Dynamics are continuous within the season and discrete between the seasons. Bottom panels show the discrete update of spore bank (turquoise) and yield (magenta) variables after each season.

We also explore how different number of break crop seasons after a single season of oilseed rape change the spore bank value (Fig. 4.5). Results show that the number of break crop seasons needed to control the infection – i.e. prevent a build-up of sclerotia in the soil – depends on the initial spore bank value. At low values of spore bank ($S(t{=}0){=}10$), a minimum of 3 break crops are needed to control the infection. However, at high values of spore bank ($S(t{=}0){=}30$), 1-year of break crop already prevents an infection build-up.

Because of farmers' interest in increasing the cropping frequency of OSR, we constraint the study of cultural control strategy in combination with genetic control and/or fungicides (CG, CF, CGF) to the application of a single season of break crop.

*Single fungicide (F) and single genetic (G) control strategies.*

**Single strategy: application of fungicides (F).** To study the application of fungicides, we simulate 20 seasons of consecutive OSR where we apply fungicide ($\mu > 0$) at the beginning of each season. The reduction of infection compared to the null strategy ∅ is caused by the reduction of all the initial inocula $i_0$. Because fungicides can have different efficiencies – depending on the drug and the dose – we study

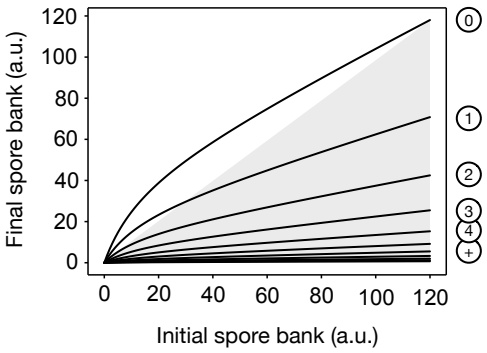


Figure 4.5: **Effect of break crops in the control of soil sclerotia after one season of oilseed rape.** The graph shows the spore bank value after one season of oilseed rape and 0 to 8 seasons of break crops, given a range of initial values of spore bank (0 to 100, in units of 1). In the shadowed area (grey), the final spore bank value is equal (diagonal) or lower than the initial spore bank value.

the range of $\mu$ in decimal values, from 0 to 1. To understand the effect, we focus on the value of spore bank and cumulative yield at the end the 20th season (Fig. ??). Results show a quick drop from $\mu$=0.6 ($S$=80.58) to $\mu$=0.9 ($S$=3.91), where there is no build-up of soil sclerotia. The spore bank drop is reflected in the yield gain. We have a yield higher than 80% for fungicide efficiency $\mu \geq 0.8$.

**Single strategy: use of a genetic resistant cultivar (G).** To study the effect of cultivating oilseed rape variants with resistance, we simulate 20 seasons of consecutive OSR diminishing the pathogen fitness for the crop ($w < 1$). This reduces the rate of infection, compared to the null strategy $\emptyset$. The maximum infected host density, at the end of the 20th season, is, in consequence, lower. Because only variants with partial resistance are known, we define $w=1-\rho$ and we explore the range of $\rho$ in decimal values, from 0 to 1. As done with the fungicides, we study the final values of spore bank and yield at the end of 20 seasons (Fig. 4.6). Results show a sigmoidal decay of spore bank along the range of values. For $\rho \geq 0.6$, the final spore bank is smaller than the initial ($S(t=20)$=3.00). The yield values show a sigmoidal increase where the final value is $>$ 80% of the maximum for $\rho \geq 0.4$.

*Integration of two control strategies: cultural-fungicide (CF), cultural-genetic (CG) and fungicide-genetic (FG) control*

**Double control by cultural rotations and fungicides (CF)**. We combine rotations of OSR-BC (1 break crop season) with the application of fungicides in the OSR seasons (Fig. 4.7). We apply the same analysis than in the single fungicide control (F), simulating 20 seasons with alternations of OSR and BC. Results show that the final spore bank is generally reduced to less than half the values obtained with the

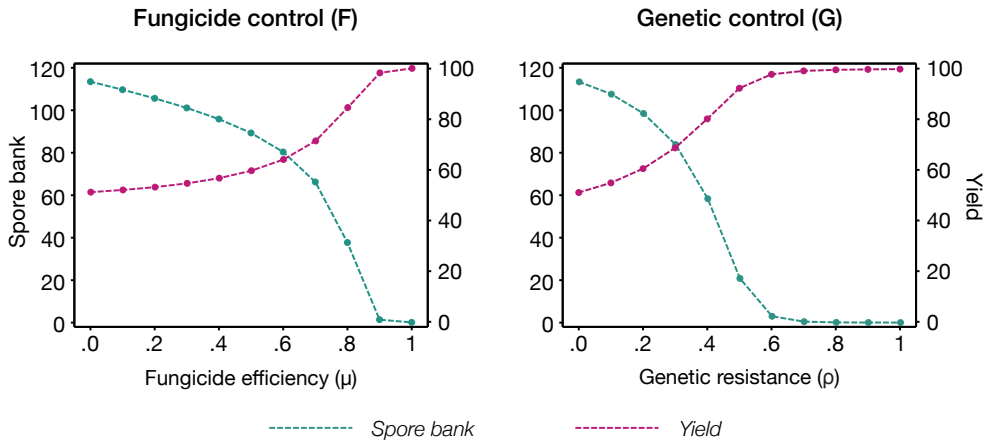


Figure 4.6: **Spore bank and yield values after 20 seasons of fungicide (F) or genetic (G) control.** In the left panel, different values of fungicide efficiency $\mu$ are explored. In the right panel, different values of genetic resistance $\rho$ are explored. In both panels, we show the values of spore bank (blue) and yield (red) after 20 consecutive season of OSR with the corresponding control strategy.

single strategy. The final spore bank is smaller than the initial for $\mu \geq 0.8$. Yield is $> 80\%$ of the maximum for $\mu \geq 0.6$, if we consider the maximum yield of reference the corresponding to OSR-BC alternations without infection.

**Double control by cultural rotations and genetic resistance (CG)**. We use rotations of OSR-BC (1 break crop season) by cultivating a variant with genetic resistance in the OSR seasons (Fig. 4.7). We apply the same analysis than in the single genetic control (G), simulating 20 seasons with alternations of OSR and BC. The sigmoidal decay is maintained, with the values reduced to less than half the values of the single strategy. The final spore bank is smaller than the initial for $\rho \geq 0.4$. Yield is $> 80\%$ of the maximum for $\mu \geq 0.3$, if we consider the maximum yield of reference the corresponding to OSR-BC alternations without infection.

**Double control by fungicides and genetic resistance (GF)**. To compare the different fungicide efficiencies $\mu$ with different levels of genetic resistance $\rho$, we do a pairwise comparison and analyse for which values the final spore bank is smaller than the initial one (no build-up of the soil sclerotia) and for which values the final yield is $> 80\%$ of the maximum (when consecutive OSR are cultivated without infection) (Fig. 4.8). Results show that in 70.25% of the combinations, there is no build-up of soil sclerotia; and in 80.17% of the combinations, the yield is $> 80\%$ of the maximum. For both, the space is more constrained by the level of fungicide efficiency than by the level of genetic resistance.

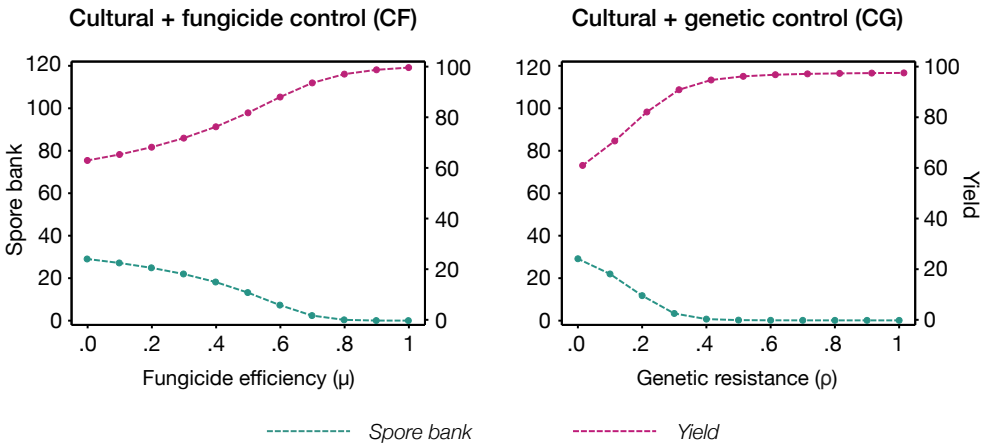


Figure 4.7: **Spore bank and yield values after 20 seasons of cultural and fungicide (CF) or cultural and genetic (CG) control.** In the left panel, different values of fungicide efficiency $\mu$ are explored. In the right panel, different values of genetic resistance $\rho$ are explored. In both panels, we show the values of spore bank (blue) and yield (red) after 20 consecutive season of OSR with the corresponding control strategy.

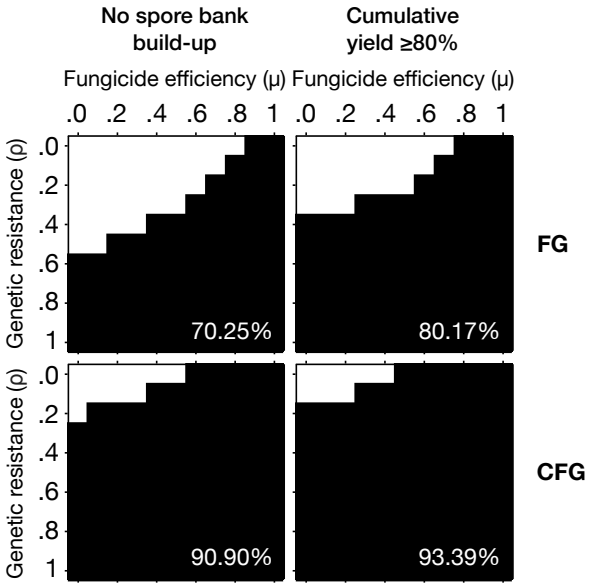


Figure 4.8: **Final spore bank and final yield qualitative values for fungicide-genetic (FG) and cultural-fungicide-genetic (CFG) control strategy.** We calculate the final spore bank and the final yield after 20 seasons of combined fungicide-genetic control (FG) – top panel – and combined cultural-fungicide-genetic control (CFG) – bottom panel –, for different values of genetic resistance ($\rho$, $y$ axis) and fungicide efficiency ($\mu$, $x$ axis). If the final spore bank is lower than the initial spore bank, there is no build-up (black); if it is higher, there is build-up (white). For the final yield, we set a threshold to 80% of the maximum yield obtained with consecutive OSR cultivation (for FG) or with OSR-BC cultivation (for CFG), both without infection; and indicate if it yields equal or less (white) or more (black) than 80%. The indicated percentages show the proportion of combinations of fungicide efficiency and resistance that allow infection control.

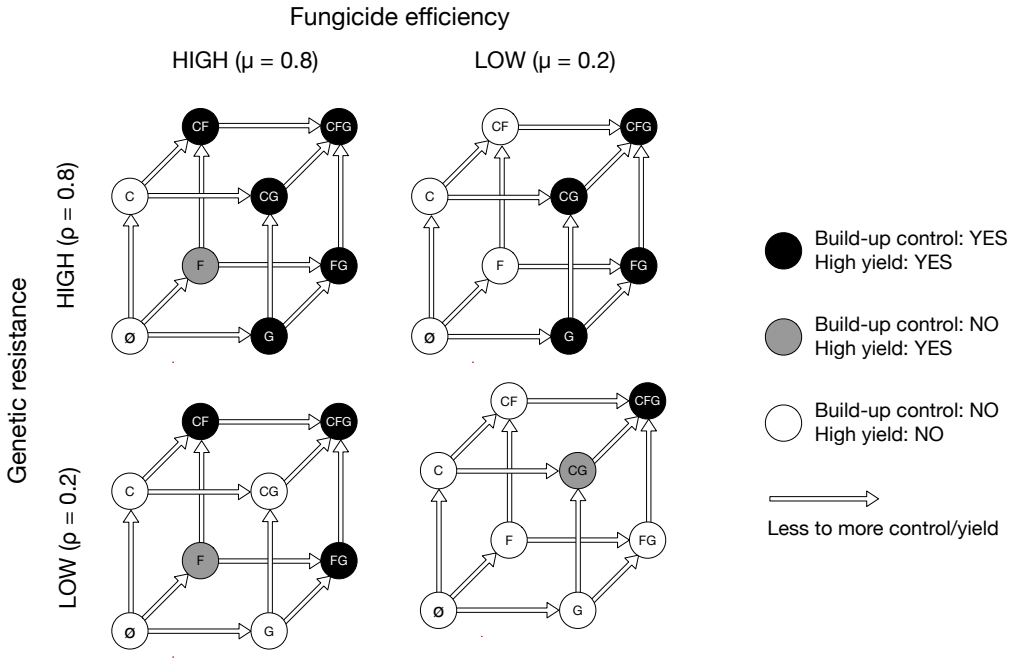


Figure 4.9: **Comparison of all control strategies for build-up control and yield.** We calculate the final spore bank and the final yield after 20 seasons for all strategies and compare if they control the build-up (i.e. the final spore bank is lower than the small one) and if they have high cumulative yield (i.e. the total yield after 20 seasons is higher than 80% of the yield when grown without infection). We compare results when fungicide efficiency is high ($\mu$=0.8) and low ($\mu$=0.2), and for high genetic resistance ($\rho$=0.8) and low genetic resistance ($\rho$=0.2). Strategies do either (i) control the build up and yield high (black), (ii) not control the build up but yield high (grey), or (iii) not control the build-up and not yield high (white). In all cubes, arrows point to strategies that perform quantitatively better.

*Integration of three control strategies: cultural-fungicide-genetic (CFG) control*

The integration of three control strategies is modelled during 20 seasons in which (1) we use rotations of OSR-BC (1 break crop season), (2) we apply different values of fungicide efficiency $\mu$, and (3) we set different values of genetic resistance $\rho$ for the OSR crop. To study the effect of combining the three strategies, we do a pairwise comparison of values of $\mu$ and $\rho$ (as in the FG strategy) but for simulations in which OSR and BC have been alternated (instead of consecutive OSR) (Fig. 4.8). Results show that in 90.90% of the combinations, there is no build-up of soil sclerotia; and in 93.39% of the combinations, the yield is $>$ 80% of the maximum when alternations of OSR-BC are cultivated without infection. Thus, the triple combination increases the space where infection control and maximisation of yield is possible. For both goals, the space is more constrained by the level of fungicide efficiency than by the level of genetic resistance.

On the other hand, when we compare all eight strategies (∅, C, F, G, CF, CG, FG, CFG) for high ($\mu$=0.8) and low ($\mu$=0.2) fungicide efficiency, and for high ($\rho$=0.8) and low genetic resistance ($\rho$=0.2), the triple control CFG performs best in both controlling the build-up (lowering the spore bank value to less than the initial value) and having a high yield (Fig. 4.9).

## 4.5 DISCUSSION

The adaptation of the generic model presented in Bargués-Ribera and Gokhale (2020) to oilseed rape rotations with cereal break crops has allowed the theoretical exploration of integrated pest management for the control of *Sclerotinia sclerotiorum* in oilseed rape, the host. In the study, we have modelled a soil spore bank – corresponding to the number of soil sclerotia – as a new feature, and we have extended the control practices to include host genetic resistance and the application of fungicides. The results presented in this chapter are a first step on the exploration of integrated management for SSR, as the parameters should be adjusted more accurately to the data to extract final conclusions. The following are a series of next steps that should be implemented for the future applicability of the results:

**Parametrisation using an optimisation algorithm.** Given a set of field data that relates to our model variables, we can find parameter values that approximate our simulations to reality. As discussed in methods, we can relate, for example, the stem rot index with the percentage of infected host density, as both are indicators of percentage of crop damage. Other indications such as the number of apothecia in a field could help to estimate the spore bank status, and long term studies with consecutive seasons of OSR could help to adjust the build-up of the infection. To do the parametrisation, we would use an optimisation algorithm that, given a range of possible values, would find the one for which simulations fit the data the most, providing more reliability to our results.

**Cost assessment of strategies**. In the model presented, we do not include any costs for the control strategies. For cultural control, the immediate cost is the reduction of cropping frequency of OSR; however break crops are cereals of commercial interest which can provide other benefits. On the other hand, spraying fungicides is not always cost-effective: farmers are recommended to spray only if a yield loss higher than 25-30% is expected, for the economic costs associated to the product and its application (Dunker and Tiedemann, 2004). Also, a cultivar with genetic resistance can carry yield penalties, associated with crop traits such as the number of pods per plant or seeds per pod, which can vary due to resource reallocation (Brown, 2002). Including the costs can change the optimality of the strategies, as the eco-

nomic costs of, for example, the triple control CFG, could be higher than its benefits.

**Study of pathogen evolution.** As mentioned, we have assumed constant or durable effectivity of fungicide application or genetic resistance. However, this disregards pathogen evolution. *Sclerotinia* resistance to fungicides is rare, due to its monocyclic life cycle (Derbyshire and Denton-Giles, 2016). However, resistance has been reported in a few cases which had a ten-year application of fungicides (Gossen et al., 2001). Pathogen virulence evolution for cultivars with partial resistance could evolve as well, lowering the effectivity of the genetic control (see 'Perspectives').

Overall, the study shows the potential of the generic model for further investigations that are of interest for the farmers and the agronomic sector.

# 5

# PERSPECTIVES

The two research projects presented in this part of the thesis investigate the effect of crop rotations and the integration of multiple control methods in the situations addressed in their description. In this chapter, I present an outlook on further scenarios to explore using the models, and I explain how this exploration could be carried out. My focuses are pathogen evolution and the extension of the number of strategies studied. Other possibilities are discussed briefly.

## 5.1 INSIGHTS ON PATHOGEN EVOLUTION

In the first research project (Chapter 3), I modelled pathogen virulence evolution by allowing an initial strain to transition to mutant strains with a fitness advantage; the consequent increased growth rate provoked more damage to the host, corresponding to more virulence. In the second research project, I ignored pathogen evolution and focused on the interaction of a single pathogen strain with the host crops. This included a crop variant with genetic resistance, which translated as a fitness disadvantage for the pathogen.

Here, I formalise a proposal for pathogen evolution which combines the methods from Chapter 3 with mathematical models from previous literature. In the proposal, the pathogen can adapt to a single host variant (specialist pathogen) or it can extend the host range (generalist pathogen). For extending the host range, I focus on the gene-for-gene model (Flor, 1956), used in plant-pathogen interactions, and I adapt mathematical formalisations from Agrawal and Lively (2002) and Song et al. (2015), which study the continuum between gene-for-gene and matching alleles models.

### *Gene-for-gene vs. matching alleles models of infection*

In his paper in 1956, Flor studied virulence evolution of flax rust (Flor, 1956). Crossing different flax rust races, he observed that virulence was, in most cases, a recessive character. Also, that for each resistance gene in the host, there was a gene conditioning pathogenicity in the parasite. He described this complementary interaction of genes as gene-for-gene relationship. However, there was no limit in the number of genes for virulence in the parasite; so parasites with virulence for

multiple hosts could occur.

Thus, in the gene-for-gene model, one pathogen genotype can have a broad host range ('universal virulence', or here, generalist pathogen). This has been contrasted to the matching alleles model, where an exact genetic match from both host and parasite is required for infection, as in self/non-self recognition systems of invertebrates (virulence is not universal, the pathogen is a specialist) (Agrawal and Lively, 2002). While the matching alleles polymorphisms are easily maintained by negative frequency dependent selection, the gene-for-gene model requires costs of virulence to keep the generalised genotype from going to fixation.

In their paper, Agrawal and Lively studied the continuum between gene-for-gene and matching alleles using a mathematical model in which two loci with two alleles each were considered (Agrawal and Lively, 2002). Later, Song et al. (2015), adapted the model to allow for changes in population sizes using the Lotka-Volterra equations. Here, I propose to modify the pathogen fitness value in our equations (determined by $W_{ji}$) according to the costs of resistance $\kappa$ and the value in the continuum between the gene-for-gene and matching alleles model $\alpha$, as in their models. This allows some of the pathogen strains ($P_2$) to be infective (virulent) for a host crop variant with resistance ($H_2$), while specialist strains ($P_1$) only infect the susceptible host ($H_1$):

| | $H_1$ | $H_2$ |
|---|---|---|
| $P_1$ | $w_{ji}$ | $w_{ji}$ (=0) |
| $P_2$ | $\alpha(w_{ji} - \alpha\kappa)$ | $w_{ji} - \alpha\kappa$ |

When $\alpha$=0, the model follows a pure matching alleles relationship; when $\alpha$=1, the model follows a pure gene-for-gene relationship.

The initial avirulent specialist strain ($P_1$) does transitions to strains which have an increased fitness in the susceptible host variant (other $P_1$ specialists, with $w_{ji}$=0 for $H_2$), and to strains which have some fitness in both susceptible and resistant host variants with a cost ($P_2$ strains, with $w_{ji}$>0 for both $H_1$ and $H_2$) (Fig. 5.1a).

In Fig. 5.1 I show an arbitrary example of pathogen eco-evolutionary dynamics in the middle point of the continuum ($\alpha$=0.5) when seasons of the resistant cultivar are only grown when the susceptible crop has been cultivated previously in the field, with alternations with non-host crops. Specifically, the pattern is: 4 seasons of break crop, 2 seasons of susceptible host crop, 1 season of break crop and 3 seasons of resistant host crop (Fig. 5.1b). If we track the variations of frequency of pathogen strains, we see that when the susceptible crop is cultivated, virulent

strains of the specialist pathogen increase in frequency (Fig. 5.1c, seasons 4 and 5). During the break crop season, frequencies do not change (Fig. 5.1c, season 6). When the crop with genetic resistance is cultivated, the generalist strains take over the specialist strains of pathogen (Fig. 5.1c, seasons 7, 8 and 9).

The curve by which the generalists take over the specialist strains changes depending on the value of $\alpha$ (Fig. 5.1). When the infection model corresponds to matching alleles ($\alpha$=0), the curve is steeper; i.e. transition is faster. When the infection model corresponds to pure gene-for-gene ($\alpha$=1), the transition is slower.

*Other considerations for pathogen evolution*

Although the gene-for-gene relationship is commonly used by plant pathologists, it mainly applies to host resistance through major R genes. Resistance can come from other mechanisms, such as adult plant resistance or minor R genes. Adult plant resistance, for example, is also controlled by the action of single genes, but resistance is not mediated by an effector-receptor interaction process, which makes it more difficult for the pathogen to overcome it (Burdon et al., 2014). Regarding our example case, *Sclerotinia* of oilseed rape, pathogenicity is related to the production of oxalic acid. Then, host resistance can be enhanced by overexpressing the oxidase enzymes of the plant metabolism (Dong et al., 2008). These examples show that the modelling of pathogen virulence evolution has to be adapted to the case study of interest.

For the *Sclerotinia* example, there is an additional factor that should be taken into account: *Sclerotinia* is a monocyclic pathogen, which reproduces once per season through a primary inoculum. This feature has an impact on the rate of evolution of the pathogen: mutations can only occur in the generation of this primary inoculum, not throughout the season, and this diminishes the risk of developing resistance (Grimmer et al., 2015). The modelling framework for pathogen evolution presented corresponds to polycyclic pathogens, which reproduce and mutate within the season. To study evolution of monocyclic pathogens, we could include mutant pathogen strains at very low frequencies in the initial inoculum, which would vary in frequency depending on their fitness; or allow mutations, with a certain probability, at the beginning of each season.

On the other hand, I have focused on pathogen evolution to overcome host resistance, but the evolution of fungicide resistance – taking into account factors such as the dose of fungicide applied (Mikaberidze et al., 2017) – should also be included. Overall, the pathogen fitness matrix is a useful tool to model pathogen evolution.

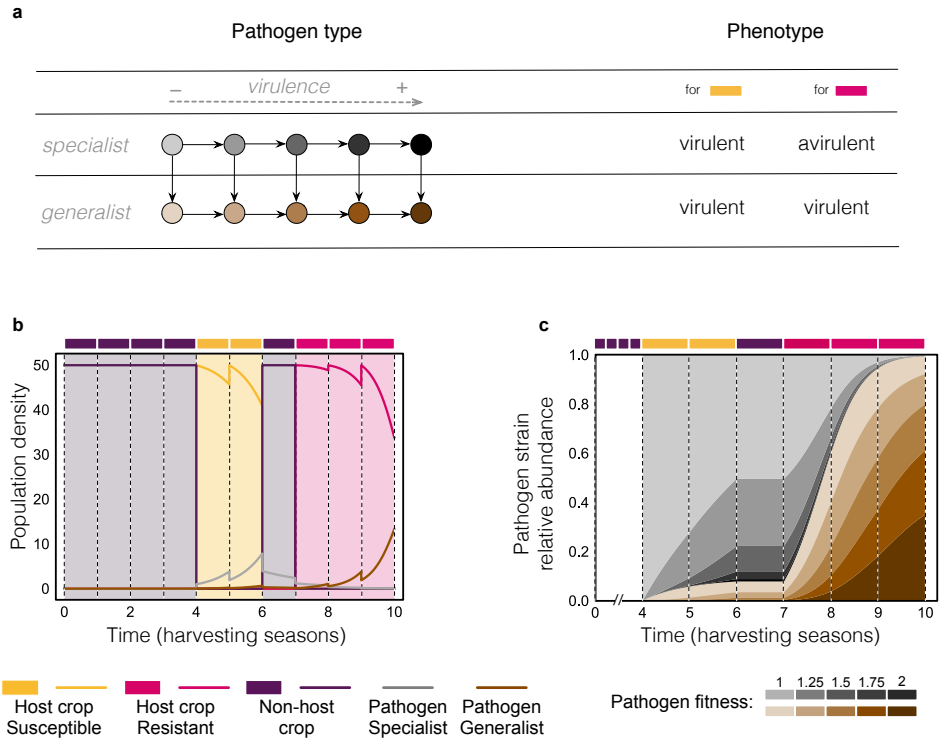


Figure 5.1: **Pathogen virulence evolution to a host resistant crop: description and dynamics.** A) Description of pathogen types – specialist (grey) or generalist (brown) – and their interactions with the host – virulence or avirulence. Multiple strains of each type exist, with different values of pathogen fitness, which relates to more virulent interactions with the host. B) Population dynamics of host and pathogen for the indicated rotation pattern. All pathogen strains are present, grouped according to specialist or generalist types. C) Relative abundance of pathogen strains during the seasons with infection. Initially, only the specialist strain with fitness $w_{ji}$=1 is present, but it mutates at rate $\mu$=0.1 into neighbour strains, which increase on frequency depending on their fitness and interaction with the host. In this example, $\alpha$=0.5.

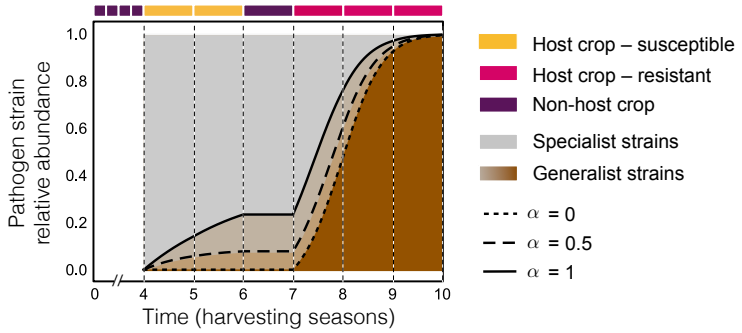


Figure 5.2: **Relative abundance of pathogen strains according to the value of $\alpha$.** When $\alpha$=0, it corresponds to a matching alleles model; while when $\alpha$=1, it corresponds to a gene-for-gene relationship. The example shown corresponds to the one in Fig. 5.1c; but we group the pathogen strains in specialists and generalists, and we simulate the same scenario with different values of $\alpha$.

When applied to a case study, the mechanisms of host resistance and pathogen virulence and reproduction must be considered to adapt the model to the biology.

## 5.2 EXPANDING THE COMBINATORIAL SPACE

In the first research project, I defined two types of crop. But, as discussed, I could define more; either by changing their soil and cash contribution ($\beta_i$) or by changing their interaction with the pathogen. In the second project, I have 8 strategies, in which the seasonal pattern is regularly defined. However, I could combine these strategies in time, with irregular patterns (as in the pathogen evolution example), giving more possibilities. All of this increases the combinatorial space and the number of management strategies. Because of computational constraints, it would be time-consuming to do an exhaustive search. Thus, I propose to use an optimisation algorithm to find which strategy maximises the variable of interest (i.e. yield, economic benefit or minimisation of infection) efficiently.

### *An example with simulated annealing*

An example of optimisation algorithm that could be used is simulated annealing. The simulated annealing algorithm is inspired by the annealing technique in metallurgy, where a material is first heated above a certain temperature and then cooled under control to improve crystallisation (Kendall, 2000; Kirkpatrick et al., 1983). In the optimisation algorithm, the search in the solution space starts at high temperature and it cools down with the iterations. The search starts with a random initial solution (sequence) and the neighbours (where only one element changes) are explored. The temperature is used to regulate the probability of accepting worse solutions: higher at the initial iterations to extend the search, lower at the end, to reach the global optimum.

Simulated annealing would be appropriate because it has a discrete number of solutions that would correspond to our number of possible sequences. Also, its ability to avoid falling into local optima would assure to find the rotation pattern that best fits our goal. In the following page I indicate the steps of the algorithm in a pseudocode example (Fig. 5.1).

I have tested an implementation of the simulated annealing algorithm based on integrated pest management. The function to be optimised calculates the balance between costs (e.g. economical costs) and benefits (e.g. yield) for rotation sequences of four strategies: susceptible and resistant variants of a crop, with or without fungicide application. Table 5.1 proposes cost-benefit values for each strategy, in

Table 5.1: **An example of cost-benefit balance values for strategy evaluation.** Each strategy is assigned a benefit, according to the yield, and an economic cost. Resistant crops have a yield penalty, and the application of fungicides is costly. The balance rests the costs to the benefits.

| Strategy | Benefit | Cost | Balance |
|---|---|---|---|
| Susceptible crop, fungicide: no | 1 | 0 | 1 |
| Resistant crop, fungicide: no | 0.9 | 0 | 0.9 |
| Susceptible crop, fungicide: yes | 1 | 0.25 | 0.75 |
| Resistant crop, fungicide: yes | 0.9 | 0.25 | 0.65 |

absence of infection, considering a yield penalty for the resistant crop and an economic cost for the application of fungicides.

```
solution1 = random() # define a random starting solution

T = T_max  # max. temperature
T_min = T_min # min. temperature
alpha = alpha # factor by which temperature decreases at each iteration

while T > T_min: # iterations
        value1 = balance(solution1)  # calculate the balance for solution 1
        solution2 = neighbour(solution1) # find a neighboring solution
        value2 = balance(solution2) # calculate the balance for solution 2

        if value2 > value1:       # compare balance of solution 1 and 2
                solution1 = solution2  # if solution 2 is better, keep it.
        elif value2 < value1:   # if solution 2 is worst,
                # calculate a probability of acceptance
                accept = probability_function(solution1, solution2, T)
                # if probability is higher than a random 0-1 value,
                if accept > random():
                                solution1 = solution2 # keep solution 2.

         T = T * alpha  # reduce temperature

print(solution1) # show final solution
```

Figure 5.1: **Pseudocode for a simulated annealing algorithm.** An initial random solution and fixed parameter values are defined. Iterations of the algorithm are run while temperature decreases. The final solution is shown. (blue = predefined functions, green = comments)

In this simplified case, the cost-benefit balance function has a known global optimum: a rotation sequence with all seasons of susceptible crop. Because of this knowledge, I can adjust the parameter values of the algorithm and set additional iter-

ations, so that the global optimum is found. The current implementation of the algorithm takes <0.04 seconds to find the global optimum for a sequence of 20 seasons ($4^{20}$ possible strategies). It is available online at: *https://github.com/tecoevo/sclerotinia*.

By expanding this algorithm, I can assess the optimality of rotation patterns under infection, coupling infection to yield doing an adequate assessment of benefits and costs of each strategy. This can be applied to the case study of *Sclerotinia* of oilseed rape to explore complex patterns of integrated pest management. For that, I would study the market values of oilseed rape varieties and the costs associated to the spraying of fungicides. Importantly, I should take into account the value of break crops, for they provide economic gains, but exclude the cultivation of oilseed rape.

## 5.3 OTHERS

In both projects, we have focused in the exploration of one field. The model could be expanded to multiple fields, which would require the implementation of new features. Among them, I should take into account the migration of pathogens between fields (McQuaid et al., 2017), and regard herd immunity between fields with crop resistance (Milne et al., 2015).

Part III

# ECO-EVOLUTIONARY DYNAMICS OF A VECTOR-BORNE DISEASE

# 6

# INTRODUCTION TO VECTOR-BORNE DISEASE MODELLING

The application of eco-evolutionary informed pathogen management strategies is not limited to agriculture. A well-known use is the administration of drugs in humans: the drug imposes a new selective environment to the disease-causing parasite, in order to reduce or eradicate its population to improve host health. In this section, we focus on the example of malaria and its treatment strategies. As a vector-borne disease, the parasite goes through both a human host and a mosquito vector, which can exert different – and antagonistic – selective pressures during its life cycle. We study how to use this feature for transmission interruption of the disease and the management of drug-resistant mutant parasites. The study can be an example for other parasites with complex life cycles and it gives insights into the global challenge of human health.

## 6.1 CONCEPTUAL FRAMEWORK

*The role of vectors in disease transmission*

A vector is any agent which carries and transmits an infectious pathogen into another living organism which serves as host (Wilson et al., 2017). Some pathogens use vectors mechanically, i.e. without reproduction or development inside the carrier, but others have evolved life-cycles which require vectors to be completed. Pathogens which have evolved vector-based modes of transmission often have more virulence (Ewald, 1983). The reason is an optimal ratio of cost-benefit at more severity of disease: when the pathogen immobilises the host, the transmission does not decay as the vector is mobile and, behaviourally, the host might be less likely to kill the vector. The lower cost, thus, changes the trade-off by which they can exploit host resources. On the other hand, the spread dynamics of vector-borne diseases are also different: when the vector is a flying arthropod, it can travel long distances. Moreover, vector density can be very high, and multiple infections can occur within a single host (Alizon et al., 2009).

The effect of vector transmission has been included in models of infectious diseases, bringing non-linear behaviours that the host dynamics alone cannot represent (Luz et al., 2010). However, it is modelled mechanically, as an inert mean of transport. Some features of vector biology such as seasonality, age structure or vec-

tor lifespan have been considered; but not the selective pressure that the pathogen faces in the vector environment (Wilson et al., 2017). Generally, the switch between host and vector can be compared to multi-host parasites (Gandon, 2004). In consequence, we should learn from multi-host theoretical approaches to know the potential effects of vector-based transmission on the evolution of the parasite.

One of the most impactful vector-borne diseases is malaria. Malaria is caused by *Plasmodium*, a parasitic protozoon of the phylum Apicomplexa, and transmitted between vertebrate hosts – e.g. humans – by the female mosquitoes of the *Anopheles* genus. In the vertebrate host, the parasite infects erythrocytes and produce either multiple asexual parasites, able to infect other cells, or one gamete, the transmissible parasite form (Rosenberg, 2008). This trait is an example trade-off within the host explored for disease transmission (Alizon et al., 2009); but the fitness or dynamics of the transmissible form in the vector, which influences the probability transmission through the bite, have received low attention. The interactions between *Plasmodium* and the vector could influence parasite evolution and change infection dynamics.

*The past, present and future of the fight against malaria*

Malaria is widely spread in tropical and subtropical areas around the globe. As mentioned, both human host and mosquito vector are necessary for completing the life cycle of *Plasmodium*, its causing agent. In 2017, 219 million malaria cases and 435,000 malaria deaths were reported worldwide, despite it being present in the medical research agenda for more than a century (Cox, 2010; WHO, 2019a). Chloroquine was the most used antimalarial until resistance spread in the 1950s, forcing the use of combinations of drugs and alternatives such as artemisinin (Bray et al., 2005). However, new resistances continued to emerge, posing a threat to disease prevention and control still today (Hyde, 2005; Müller and Hyde, 2010).

In the current guidelines, the use of artemisinin-combination therapies is strongly recommended in most of the cases and supported by high-quality evidence for treating malaria patients (WHO, 2019a). Artemisinin is a curative treatment for the host, as it acts in the erythrocytes and can kill the parasites that infect them. Nonetheless, because artemisinin resistance exists, curative treatment can be supplemented with other strategies to facilitate the reduction of malaria incidence or, even, its eradication (Ashley et al., 2014). Among these strategies, we find approaches directed to prevention (i.e. prophylaxis), directed to the vector and directed to all the human population.

Prophylactic treatments are mainly for travellers to regions with disease: they are given before hand and act suppressing the parasite on its first stages of the life cycle. The most common prophylactics are chloroquine and the combination of atovaquone and proguanil, commercialised as Malarone (Høgh et al., 2000; Nixon et al., 2013). Strategies directed to the vector aim to reduce its population, such as insecticide-treated nets (Paton et al., 2019). When taking into account the whole human population, programs such as mass drug administration (MDA) treat a great proportion of humans to avoid disease spread and aim for the eventual eradication of the parasite, locally (Maude et al., 2009).

Plans for further malaria control take into account the role of the vector. Drugs such as primaquine that reduces infectiousness from human to mosquito are gaining interest (White et al., 2014). Importantly, the genetic modification of mosquitoes with gene drive constructs is under study with the purpose of suppressing reproduction of the mosquito, and thus, parasite transmission (Gantz et al., 2015; Hammond et al., 2016).

*Our strategy: vector biology against drug-resistant parasites to improve MDA strategies and local eradication of malaria.*

We propose a control strategy based on the experimental evidence of two of the prophylactic treatments, atovaquone and chloroquine, which drug-resistant parasites present a reproductive disadvantage respect to the wild-types in the vector stages of their life cycle (Goodman et al., 2016; Mharakurwa et al., 2013). We believe that studying the influence of vector biology in these parasite strains can help the design of successful strategies for resistance management. Particularly, we study what would happen in MDA scenarios in which these drugs are administrated. MDA has been considered for local geographical areas, such as the Great Mekong region, or isolated populations of islands (WHO, 2011). The hypothesis is that resistance would be hardly spread because of the low viability of resistant strains in the mosquitoes, and that the population of the parasite could be eradicated locally.

## 6.2 METHODOLOGICAL FRAMEWORK

To study the effect of atovaquone and chloroquine in eco-evolutionary dynamics of *Plasmodium*, the model includes the following features:

- It combines deterministic growth with stochastic sampling in different stages of the parasite life cycle.

- It predefines the interaction the wild-type and mutant pathogens with the human host and the mosquito vector using fitness values specific to the lifecycle stage.
- It works at the local life-cycle scale and at the global population scale.

The model diagram (Fig. 6.1) shows the processes which happen at the local and global scales. At both scales, the model receives inputs regarding the parasite genotypes, the size of human, mosquito and parasite populations and the drug strategy to be studied. Dynamics are run within the life-cycle and between human-mosquito populations. With the output, we can study the variation in the genetics of the parasite and the size of populations, as well as parasite extinction events.

*Discrete exponential growth for parasite reproduction (deterministic)*

The simplest form to model a population in reproduction is using the exponential growth, in which the change in time is proportional to the number of individuals in the population. In the local scale of the model, we use days as discrete unit of time, so we use the recursion equation for exponential growth:

$$n(t+1) = Rn(t) \tag{6.1}$$

In which $R$ is the reproductive factor, or number of surviving individuals per parent which replaces the population in the next time unit. The model assumes all individuals in the population are capable of reproduction. Because we use exponential growth in asexual stages of *Plasmodium* life cycle, this is a valid assumption. In our model, we adjust the value of $R$ to fit the observed experimental values in a malaria study (Rosenberg, 2008). When the effect of the drug is represented, $R \leq 0$ and the population of parasites decays.

*Multinomial sampling in population bottlenecks (stochastic)*

There are stages of the life-cycle of *Plasmodium* in which the population size is drastically reduced: only a small part of the population makes it to the next stage. Particularly, these apply to the mosquito-human and human-mosquito transmission stages, in which the bite acts as a population bottleneck. In these stages, a shift in allelic frequencies can occur due to genetic drift. Also, in the stage of sexual reproduction, the number of gametes is small and the zygotes formed depend on their random pairing, limited by the number of male gametes (as there is a female:male ratio of 4:1). In these cases, stochastic modelling offers a fairer representation of reality than deterministic models.

Here, we use the genotype frequencies as probabilities to set a multinomial distribution, from which we can sample the desired number of individuals. A multinomial distribution describes the probability of observing $K$ individuals (i.e. parasites) in each of $C$ discrete categories (i.e. number of genotypes), where the probability of observing an outcome in a category $I$ (i.e. which genotype) is $p_i$ (i.e. genotype frequency). Its formula is the following:

$$j = min\left[j' \in \{1,\ldots,k\}: \left(\sum_{i=1}^{j'} p_i\right) - X \geq 0\right]. \tag{6.2}$$

In which $j$ is one of the $k$ individuals of category $i$ if the probability $p_i$ is higher than the random auxiliary variable $X$, which is a random value between (0,1) given an uniform distribution.

For the random pairing, we use sampling without replacement to take the same number of males and female gametes. When we have the same number, we draw a probability distribution according to the genotype frequencies to obtain the same number of (diploid) zygotes.

*Pathogen fitness matrix for characterising human-parasite and mosquito-parasite interactions*

Similarly to Chapter 2, each pathogen strain (or genotype) is assigned a fitness value that modifies the growth rate in different life cycle stages. Depending on the drug environment, the fitness of a strain varies. The viability of each strain in the mosquito is also determined by a fitness matrix, which values are used as a probability of survival.

*Multi-scale modelling for the life cycle and the disease transmission*

Biological systems can be observed at different scales; from intracellular molecular interactions, to single-cell behaviour to behaviour of populations of cells. The multi-scale approach also applies to disease dynamics, in which parasite dynamics within a host can affect transmission between hosts, which has implications at the global population level (Mideo et al., 2013). Mathematical and computational modelling can integrate processes occurring at different spatiotemporal scales using nested models which link within and between-host dynamics (Mideo et al., 2008).

In our model, we focus first on the life-cycle scale, which affects later a population of hosts and vectors and the transmission between multiple populations of hosts and vectors. Thus, we represent the behaviour of the higher scale (transmission between populations) from the dynamics and interactions of the model components

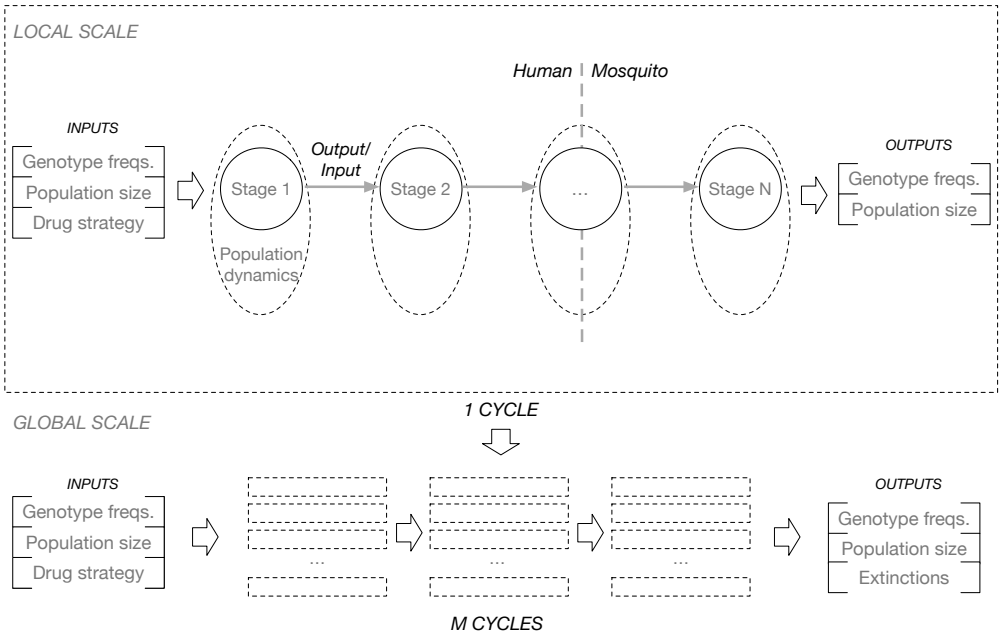


Figure 6.1: **Diagram of the model framework for eco-evolutionary dynamics of a vector-borne disease.** The model works at two scales: the local, for the parasite life cycle, and global, for transmission of the disease between human populations. Before the simulation is run, the values of parameters and variables that characterise parasite genotype frequencies, human-parasite population sizes and the drug strategy are defined as inputs, for both scales. At the local scale, parasite population dynamics are simulated for $N$ consecutive life cycle stages: the output of one stage serves as input for the next one. Stages occur either in the human host or the mosquito vector. When the simulation ends, we get a final output which corresponds to the genotype frequencies and the population size (which can diminish in the case of the parasite). At the global scale, a set of $M$ cycles occur simultaneously, in different human-mosquito individuals of a population. The output of a set of cycles serves as input for the next set, simulating disease transmission between populations.

of a lower, more detailed, scale (life-cycle). The life cycle works as a unit occurring simultaneously as many times as infections happen, and repeated temporally depending on a number of transmission events (Fig. 6.1).

## 6.3 RESEARCH QUESTION

I use the methodological framework to study the following research question:

- Do human-mosquito antagonistic selective pressures impede the spread of pathogen drug resistance under mass drug administration regimes of atovaquone and chloroquine?

The study is developed and presented as a manuscript in Chapter 7.

# 7

# ECO-EVOLUTIONARY DYNAMICS OF *PLASMODIUM* GENOTYPES UNDER MASS DRUG ADMINISTRATION

The content of this chapter has been written as a manuscript and it is available online as a preprint:

Bargués-Ribera M, Reeves RG and Gokhale CS (2019) Eco-evolutionary dynamics of *Plasmodium* genotypes under mass drug administration. bioRxiv 818039; doi: https://doi.org/10.1101/818039.

The `Python` core codes, describing the model, are available on `Github` at *https://github.com/tecoevo/MDAmalaria*.

## 7.1 ABSTRACT

Mass Drug Administration (MDA) is regarded as a potential strategy for locally interrupting transmission of human malaria under specific circumstances. However, insights on how MDA affects the eco-evolutionary dynamics of different *Plasmodium* species are not well known. We provide a computational model where the ecologically explicit life cycle of the parasite is implemented. Since the parasite inhabits two different ecological niches – human host and the mosquito – it undergoes different selection pressures during its reproduction. We use the model to perform an evolutionary analysis of the dynamics of resistance alleles under atovaquone, chloroquine and combined atovaquone-chloroquine drug treatments. Our study shows how the reduced viability of resistant parasites in the mosquito affects the spread of resistance and transmission interruption in treated human populations. Overall, results confirm that the disadvantage of drug-resistant genotypes in the mosquito vector is a good tool to achieve malaria control goals under MDA programmes.

## 7.2 AUTHOR SUMMARY

Every year there are millions of new malaria cases reported worldwide. The cause of the disease is the infection by *Plasmodium*, a protozoan which is transmitted between humans through the bite of a mosquito. Antimalarials have existed since long, but *Plasmodium* has evolved resistance to the treatment, making it necessary to develop new strategies to heal the infected humans. Lately, it has been pointed

out that mosquitoes could be our allies when using drugs such as atovaquone, which resistant parasites have difficulties to reproduce in the mosquito. Here we study the scenarios in which these drugs, used in Mass Drug Administration (MDA) programmes, can interrupt the transmission of malaria in local treated populations.

## 7.3 INTRODUCTION

Mass Drug Administration (MDA) is currently considered by the World Health Organisation as a potential strategy for locally interrupting transmission of human malaria in isolated low transmission areas (WHO, 2019b). Research studies show that resistant strains of the pathogen have a reproductive disadvantage in mosquitoes: mutant zygotes have low viability and often cannot complete their life cycle. Here we analyse how MDA programmes can help transmission interruption by reducing the parasite population size by means of both the drug effect in the humans and the interruption of the life cycle of resistant strains in the mosquito.

Anti-malarial programmes which include MDA entail simultaneously providing a substantial fraction of a human population ($> 70 - 80\%$) with courses of drugs at repeated intervals to eliminate malaria transmission in an area. Global applications of MDA to control neglected tropical diseases like onchocerciasis, schistosomiasis and lymphatic filariasis have led to major successes on regional scales (Webster et al., 2014). However, defining the impact of MDA in malaria control is hampered by limited data. The circumstances in which MDA is most likely to prove effective in achieving interrupted transmission are exacting and include factors such as adequate access to medical facilities, effective mosquito control, limited potential for reintroduction and relatively low levels of transmission (Poirot et al., 2013). As detailed in a recent article (Eisele, 2019) MDA, in combination with vector control and improved surveillance can interrupt transmission for up to 6 months. MDA with vector control has also interrupted malaria transmission for sustained periods among isolated island populations (WHO, 2015).

From the evolutionary perspective, intuitive Darwinian principles could reason that if alleles conferring resistance to drugs are already segregating in *Plasmodium* populations, their frequency will tend to increase during MDA programs that employ those drugs. The rise of resistance acts to reduce the efficacy of drugs, both in terms of treatments and prophylactic impact. However, there is no evidence that MDA strategies increase the probability of resistance alleles arising (White, 2017), beyond that resulting from other strategies of drug use.

Recent observational and experimental studies indicate that some drug resistance alleles encountered in *Plasmodia* interfere with the completion of its life-

cycle, especially the alleles for atovaquone resistance (Goodman et al., 2017). This observation has led to the proposal that such a disadvantage could be exploited to reduce the frequency of resistant genotypes ensuring that drugs in use remain effective. The consequences that this phenomenon could have on transmission rates have not been quantified.

Herein, we propose a model that can disentangle the effects of selection in plasmodia in both the mosquito and as a consequence of human drug administration. Different to previous models which tackle resistance spread focusing exclusively in the human host (Birget et al., 2017; Bushman et al., 2018; Legros and Bonhoeffer, 2016), our model analyses the role of the vector in parasite dynamics. In doing so, we focus on the drug combination of chloroquine and atovaquone-proguanil (termed simply atovaquone). This particular drug combination is chosen because, (1) estimates of mosquito viability for drug resistant alleles are available for both drugs (Goodman et al., 2017; Mharakurwa et al., 2013), (2) both drugs are inexpensive and free of intellectual property claims, and (3) this combination of drugs has been reportedly used in patients without incident (Laufer et al., 2012). In general, the model applies to any drugs with similar properties.

Atovaquone's mode of action is attributed to drug activity against the liver and pre-liver stage parasites and targets the cytochrome b protein (cytB) (Baggish and Hill, 2002; Nixon et al., 2013). The mitochondrial genome encodes the cytB gene and a single point mutation confers a high level of drug resistance (Siregar et al., 2008). However, the parasites which carry this resistant allele are reported to be unviable in the sexual stage of the parasite occurring in the mosquito, resulting in a promising mechanism for resistance management (Goodman et al., 2017; Goodman et al., 2016). Chloroquine acts during the erythrocytic growth of the parasite (Ginsburg et al., 1999; Sullivan et al., 1998). Mutations of the nuclear-encoded Chloroquine Resistance Transporter gene (PfCRT) play a significant role in the widespread global resistance to this drug and other members of the antifolate class (Sidhu et al., 2002). Parasites with the resistant allele K76T have been reported to have an up to 9-fold fitness reduction compared to the wild-type in the mosquito life cycle stage (Mharakurwa et al., 2011, 2013). This disadvantage, however, has not stopped the spread of chloroquine resistance in the field (Mita et al., 2009; Payne, 1987; Sa et al., 2009).

Using our model, we compare the effects of three drug regimes: atovaquone, chloroquine and their combination. We study populations under MDA and in lower drug population coverage scenarios. *Plasmodium* genotype frequencies, population size and life cycle stage of extinction are tracked in the course of transmission events between uninfected human populations. We are interested in exploring how

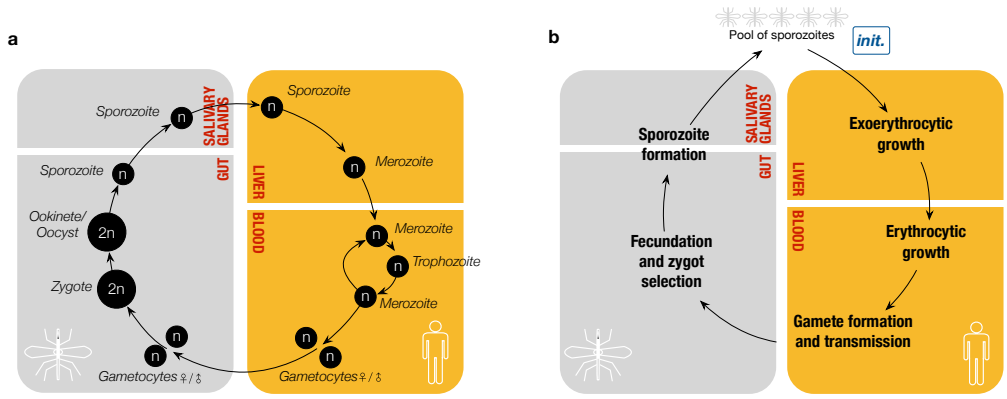


Figure 7.1: **Life cycle of *Plasmodium* in human and mosquito hosts**. a) Haploid ($n$) and diploid ($2n$) phases of the life cycle in human (yellow) and mosquito (grey) hosts. The mosquito bites the human and injects sporozoites, which are directed to the liver. Once there, they will form schizonts, which will release merozoites. These merozoites will go into the blood stream and infect erythrocytes. In the erythrocytes, merozoites will mature into trophozoites and form schizonts which will release merozoites again. Some of these merozoites will create gametocytes, which will be females or males in a ratio 4:1. The mosquito, when biting the infected human, will take some gametocytes, and in its gut fecundation occurs followed by consequent zygote formation. The zygotes which turn out to be viable will develop into motile ookinetes, which will attach to the gut wall, mature into oocyst, and release sporozoites. These sporozoites will migrate to the salivary glands, ready to be injected again in the human host in the next cycle. b) Simplified steps of life cycle in human (yellow) and mosquito (grey) hosts. For modelling purposes, we consider the life cycle to start from a pool of sporozoites which comes from the multiple mosquitoes which can bite the human. Then, the steps followed are: (i) exoerythrocytic growth, (ii) erythrocytic growth, (iii) gamete formation and transmission, (iv) fecundation and zygote selection, and (v) sporozoite formation. After these phases, the sporozoites formed become part of the initial pool of sporozoites and the cycle starts again.

the single and combined drug regimes perform in (1) managing already segregating resistance genotypes and (2) reducing the number of generations required to achieve local transmission interruption reliably. Our results show that MDA is effective at interrupting the parasite transmission and especially under atovaquone or combined atovaquone-chloroquine treatments due to the viability disadvantage of the resistant parasites.

## 7.4 MODEL

Computationally, we compartmentalise the life cycle of the parasite and implement it into a mechanistic model according to its biological stages: exoerythrocytic growth, erythrocytic growth, gamete formation and transmission, fecundation and zygote

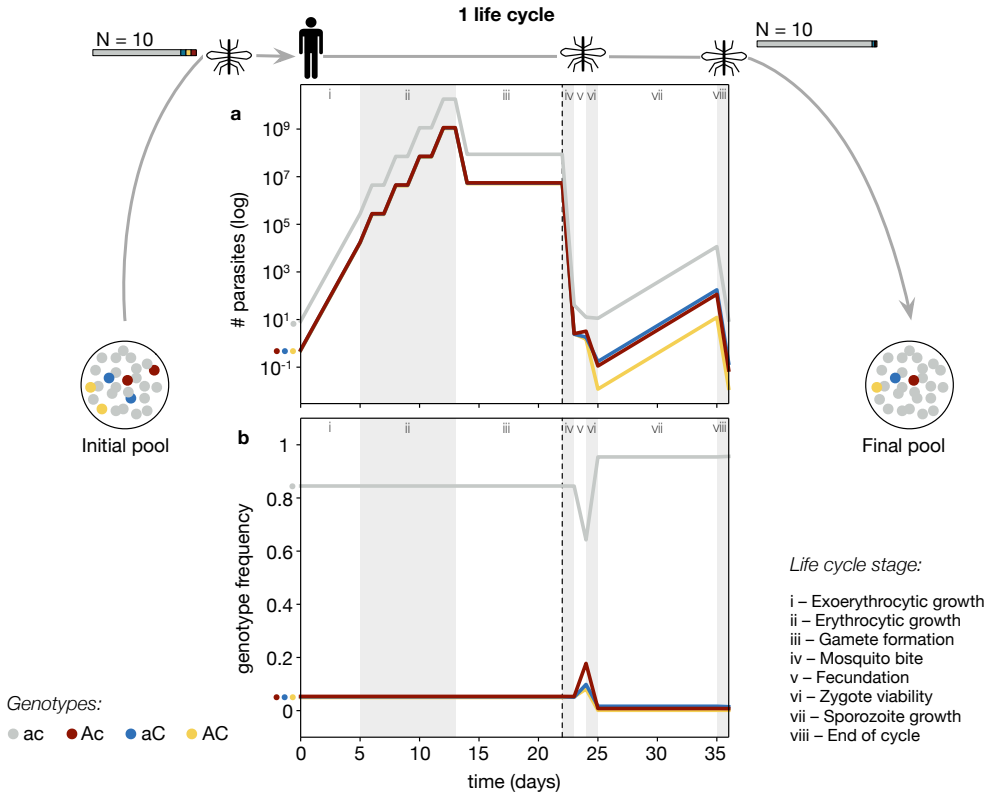


Figure 7.2: **Eco-evolutionary dynamics within one life cycle without drug selection (mean of 100 realisations)**. From the initial pool, a mosquito carrying a sample of $N$=10 parasites bites the human, starting the life cycle. Parasites follow the $i - iii$ life stages in the human host and $iv - viii$ in the mosquito (dotted line separates human-mosquito transition). At the end, after 36 days, the pool is updated with the outcome of the cycle, which corresponds to $N$=10 unless there is extinction. a) Number of parasites according to genotype – ac (grey), Ac (red), aC (blue), AC (yellow) – during the life cycle, in log scale. b) Genotype frequencies during the life cycle.

selection and sporozoite formation (Fig. 7.1). This allows us to separate processes in the host and the vector and apply different selective pressures for resistant and susceptible parasite in different compartments. The parasite reproduces in each compartment, in discrete time, with exponential growth and multinomial sampling. Eco-evolutionary dynamics occur at two levels: within-cycle (Fig. 7.2), following each stage in days as a time unit, and between multiple simultaneous cycles, referred as population transmission events (Fig. 7.3). After each transmission event, parasite population is updated. Details of the computational and mathematical implementation are explained in the Methods section.

We assume an isolated island with a constant population of 100,000 mosquitos (no seasonality), reproducing in synchrony without overlapping generations. Each mosquito is infected with parasites in the sporozoite form, ready to be injected to

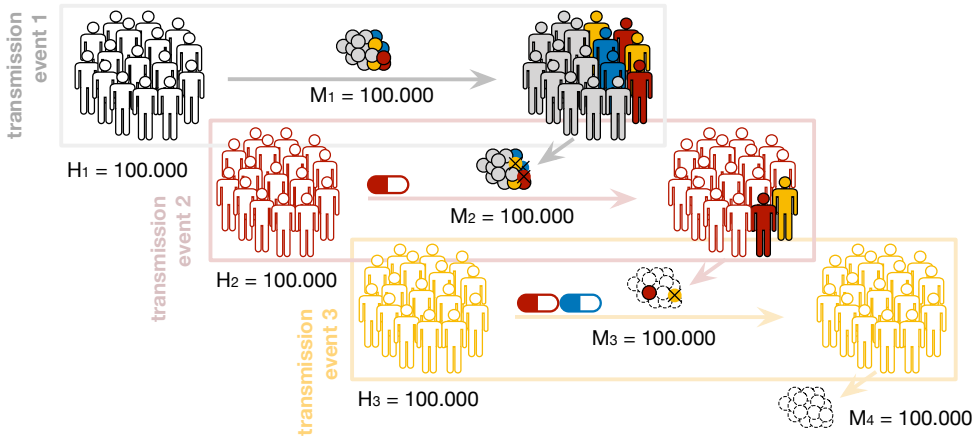


Figure 7.3: **Infection of new human populations in the course of transmission events**. In the first transmission event, the initial pool of plasmodia is carried by a population of mosquitoes $M_1$. Each mosquito bites a human from a naive human population $H_1$, which gets infected. The parasites finish their life cycle when a second population of mosquitoes $M_2$ bites the infected humans, with some parasite extinctions due to low viability. In a second transmission event, $M_2$ bites a new human population $H_2$. In this example, $H_2$ has been treated with atovaquone, which prevents some of the individuals from sustaining infection. A third population of mosquitoes $M_3$ bites $H_3$: the mosquitoes which bite non-infected humans become non-carriers, and extinctions due to low viability may occur. In this example, none of the individuals are bitten with a double-resistant parasite, thus none gets infected. The last population of mosquitoes $M_4$ bites an uninfected population of humans, and disease transmission is interrupted.

human hosts. The initial sporozoite rate, percentage of female mosquitoes with infective sporozoites in their salivary glands, is assumed for simulations to be 100%. This exceeds all field estimates from even very high transmission areas which rarely peak above 15%. However, we chose this maximal value as it provides a better appreciation of the model dynamics across the full range of possible values.

We do not model resistance allele emergence but instead assume that resistance alleles for both drugs in question already exist at appreciable frequencies in the *Plasmodium* population (0.1 each). This could realistically arise where one or both of the drugs were used in the area before MDA intervention. For one of the drugs, atovaquone, resistance is conferred by an allele of the maternally inherited mitochondrial genome (Goodman et al., 2017), while the second drug, chloroquine, is via mutations in the nuclear genome (Sidhu et al., 2002). This dictates that during segregation of both alleles during sexual reproduction double resistant genotypes are generated at a much higher frequency than if recombination between nuclear alleles was necessary.

While the viability of resistant alleles in mosquitos is the phenomena we are most focused on elucidating the impact of, we have relaxed the intensity of the effect relative to the published estimates. This allows for the possibility that published estimates may represent the more extreme end of possible values for viability in the field. Consequently, for illustration of the model, the viability in mosquitoes of atovaquone resistant alleles is increased to 0.05 (from non-viable in Goodman et al., 2017), while for chloroquine-resistant alleles viability is set to 0.3 (see SI).

We do make the simplifying assumption that each human bitten by mosquitoes is initially uninfected regardless of their history or whether they are subject to drug treatment. The percentage coverage of humans administered is studied with the values 25% (low), 50% (medium), 75% (high) and 100% (full), where high and full coverage are considered MDA scenarios. Given the set of parameters and the justifications for their ranges, we proceed with the computational model. We estimate quantities of interest such as the extinction of the parasite, the rise of resistance and the impact of the drug courses when administered with different population coverage.

## 7.5 METHODS

We compartmentalise the life cycle of the parasite according to its biological stages and implement it into a mechanistic computational model (Fig. 7.1). The parasite reproduces in each compartment, in discrete time, with exponential growth and multinomial sampling. Eco-evolutionary dynamics occur at two levels: within-cycle, following each stage in days as the time unit, and between multiple simultaneous cycles, referred as population transmission events. Importantly, all resistant genotypes are present in the population from the beginning and mutation is not considered.

### *Null-model: a cycle in absence of drug selection*

Host-vector transmission and parasite growth happen continuously in mixed populations of humans and mosquitoes carrying *Plasmodium*. In our model, the life cycle starts with a mosquito-to-human transmission of the parasite. Then, the cycle splits into stages, which work as sequential compartments receiving a parasite input and return an output that goes on, until the cycle ends. Genetically, we define a mtDNA haploid locus for atovaquone resistance with the resistant $A$ and susceptible $a$ alleles. A nDNA locus for chloroquine resistance is haploid in the asexual phase and diploid in the sexual phase. The resistant $C$ and susceptible $c$ alleles are for chloroquine. For those stages in which the parasite is haploid, the parasite

vector contains $n_i$, whereas for those in which is diploid, contains $z_i$ of the possible combinations shown below:

| | $C$ | $c$ |
|---|---|---|
| $A$ | $n_1$ | $n_3$ |
| $a$ | $n_2$ | $n_4$ |

| | $CC$ | $Cc$ | $cc$ |
|---|---|---|---|
| $A$ | $z_1$ | $z_3$ | $z_5$ |
| $a$ | $z_2$ | $z_4$ | $z_6$ |

The total number of parasites as input and output for each compartment has been adjusted to the values shown in (Rosenberg, 2008), in which the author reviewed and summarised quantitative data from different malaria studies. Dynamics within one cycle are shown in Fig. 7.2.

*Initialisation ($t$=0)*

The cycle starts when the mosquito bites and injects sporozoites in a naive human host. A sample of $N$=10 sporozoites is sorted by multinomial sampling according to the initial frequencies $f_0(n_i)$=$\{0.05, 0.05, 0.05, 0.85\}$ of the $i$ genotypes $\{AC, aC, Ac, ac\}$ where the wild type is the most common genotype.

*Exoerythrocytic growth (Day 0 to 5)*

Once in the human host, sporozoites are directed into the liver, where they form schizonts and release merozoites into the blood. For sporozoites to reproduce, we use exponential growth in discrete time:

$$n_i(t+1) \quad = \quad r_i(j,k)n_i(t) \tag{7.1}$$

where $n_i$ is number of haploid genotype $i$ with $r_i(j,k)$ as its growth rate. This equation is used in all life cycle stages, that involve asexual reproduction. The growth rate of the genotype $i$ depends on the life cycle stage $j$ and the drug scenario $k$. Without drug selection, all genotypes $i$ have the same growth within each stage $r_i(j,k)$=$b_j$. During the exoerythrocytic growth, $b_j$=5, so each sporozoite replicates into five sporozoites per day. The population is updated until $t$=5 (Rosenberg, 2008).

*Erythrocytic growth (Day 5 to 13)*

In the erythrocytic phase, schizonts generate merozoites which infect erythrocytes in the blood. Schizonts replicate in four cycles of two days. For reproduction of the parasite in this stage, we use exponential growth (Eq. 7.1). Here population is updated every two time-steps ($n_i(t+2)$) until $t$=13 with a birth rate $b_j$=16 (Rosenberg, 2008).

*Gamete formation and human-mosquito transmission (Day 13 to 23)*

Gametocytes are produced with an efficiency of $\epsilon$=0.0048 (Rosenberg, 2008). Thus, the population update is the product of the current population and the efficiency rate:

$$n_i(t+1) = \epsilon n_i(t). \tag{7.2}$$

Since the population size is large, stochastic effects are ignored, and genotype frequencies do not vary. However, gametocytes are the first step of the sexual reproduction in *Plasmodium*: female and male. The female:male ratio of gametocytes is 4 : 1 (Rosenberg, 2008), an important feature for fecundation. To implement sex differentiation, we multiply our population per ratio proportion:

$$N^f = 0.8N \tag{7.3}$$

$$N^m = 0.2N \tag{7.4}$$

Consequently, our parasite population consists of eight types depending on sex and genotype. For these gametocytes to mate, a mosquito needs to bite the infected human and pick up both male and female gametocytes, as the fecundation takes place in the mosquito gut. The mosquito bite takes typically $N$=48 gametocytes (Rosenberg, 2008) implemented through multinomial sampling. This process introduces an ecological bottleneck for the parasite population, with drift affecting the genotype frequencies.

The parasite merozoites continue to grow and infect erythrocytes causing the disease. However, here, we keep our focus on the parasite forms that follow the complete life cycle.

*Fecundation and zygote selection (Day 23 to 25)*

In the mosquito gut, gametocytes form zygotes. Male gametocytes are the limiting factor due to the biased sex ratio. We implement sampling without replacement to match male and female gametocytes, and we obtain $Z$=$N^m$ diploid zygotes, classified in six genotypes. Importantly, in the zygote genotypes, the atovaquone locus remains haploid and corresponds to the female allele, following mtDNA maternal inheritance.

The resulting zygotes $Z$ are subject to viability selection. These probability of surviving are determined as $p_i$=$\{0.015, 0.3, 0.015, 0.3, 0.05, 1\}$, corresponding each value to the diploid $z_i$ genotype. The values of $p_i$ are set considering that (a) single atovaquone resistance ($z_5$) has a very low probability of survival of $p_5$=0.05, (b) chloroquine resistance is dominant in heterozygosis ($z_2$=$z_4, z_1$=$z_3$), (c) single chloroquine resistance ($z_2, z_4$) has a fitness disadvantage respect the wild-type ($z_6$) of $p_2$=$p_4$=0.3 , and (d) the double resistant is assumed to have a multiplicative

fitness disadvantage ($z_1, z_3$). For more details on the chosen values, see Supplementary Information.

*Sporozoite growth and end of cycle (Day 25 to 36)*

The zygotes in the mosquito gut mature into oocysts and ookinetes progressively, finally forming sporozoites and going back to haploid asexual form. In the model, the mapping from diploid to haploid follows:

$$n_i = xz_i + yz_{i+2} \tag{7.5}$$

where $\{x, y\}$=2 for the matching homozygote and $\{x, y\}$=1 for the matching heterozygote (e.g. $n_{AC}$=$2z_{ACC}$+$z_{ACc}$). After the haploid conversion, the population of parasites grows exponentially following Eq. 7.1. For sporozoite formation, parasites reproduce with $b_j$=2 between $t$=25 and $t$=35. One-fifth of the sporozoites makes it to the salivary glands ($N$=0.2$N$), and finally, a sample of $N$=10 parasites is randomly selected. This sample updates the parasites carried by the mosquito, thus closing the life cycle.

*Simultaneous infections in presence of drug selection and transmission events*

In a population where malaria is present, there are multiple simultaneous infections. In the model, we simulate multiple simultaneous life-cycles within a transmission event. The number of simultaneous cycles is equivalent to the number of humans in the population, which in turn is equivalent to the number of mosquitoes. The entire population of parasites is conceptualised as a pool of sporozoites carried by the mosquitoes. In this pool, Plasmodia are distributed in groups of $N$=10, representing the infected mosquitoes. Initial pool size is one million (100.000 mosquitoes carrying $N$=10 parasites each). In absence of extinctions, pool size is maintained along the transmission event updates. During one transmission event, each mosquito bites one naive human of the population with the $N$=10 parasites. After gametocyte formation, each human host is bitten by a new uninfected mosquito to proceed with fecundation and finish the life cycle with $N$=10 parasites ready to be injected to a new human. In the results presented, there are ten transmission events, corresponding to approximatively one year if we consider the life cycle length of 36 days defined in this model.

*Drug treatment*

At the appropriate stages in the life cycle, we introduce atovaquone and chloroquine. Atovaquone affects the parasites before and during the liver stage, that is, during the exoerythrocytic growth. Chloroquine acts on the intra-erythrocytic parasites, that is, during erythrocytic growth. As in these stages, reproduction follows

discrete exponential growth; we can include the drug as suppressed growth rate for the susceptible genotypes.

The growth rate $r_i(j,k)$ defined in Eq. 7.1 can be described now as:

$$r_i(j,k) \quad = \quad b_j - (1 - w_i(j,k))2b_j \tag{7.6}$$

where in the presence of drugs the fitness of genotype $i$ is $w_i(j,k)$=0.1 if the $i$ is susceptible to drugs and $w_i(j,k)$=1 if it is resistant. Consequently, during treatment, the growth rate of susceptible genotypes becomes negative, indicating drug effectiveness. The susceptible genotypes have their fitness decreased exclusively in the stage where their nemesis drug is active, even in the case of double treatment.

*Drug population coverage*

The model allows the study of different drug population coverages by using the drug fitness disadvantage in the desired proportion of human-mosquito life cycles. The rest of life cycles are affected only by the viability selection of zygotes in the mosquito.

*Extinctions*

We consider extinction the scenario in which the initial inoculum of $N$=10 parasites is reduced to $N$=0, either in the human or in the mosquito. In the absence of drugs, the parasite can go extinct in the mosquito: under viability selection, all zygotes except for the wild-type genotype have a probability of survival less than 1, meaning that if by chance there is no wild-type, the whole population can die out. Also, if by stochastic means the gametocytes taken by the mosquito are from the same sex, there is no zygote formation and thus extinction. On the other hand, drug administration causes the death of the susceptible parasites in humans. If the corresponding resistant strain is present in the initial inoculum, there is no extinction in the human host.

## 7.6 RESULTS

Ten consecutive transmission events (non-overlapping mosquito generations) are simulated for different treatment scenarios (drug regime and population coverage). After each transmission event we record the genotype frequencies (evolutionary dynamics), size of the population of parasites (ecological dynamics) and the number of extinctions in humans and mosquito vectors. We perform 100 realisations of the complete process to account for the stochasticity in the parasite lifecycle.

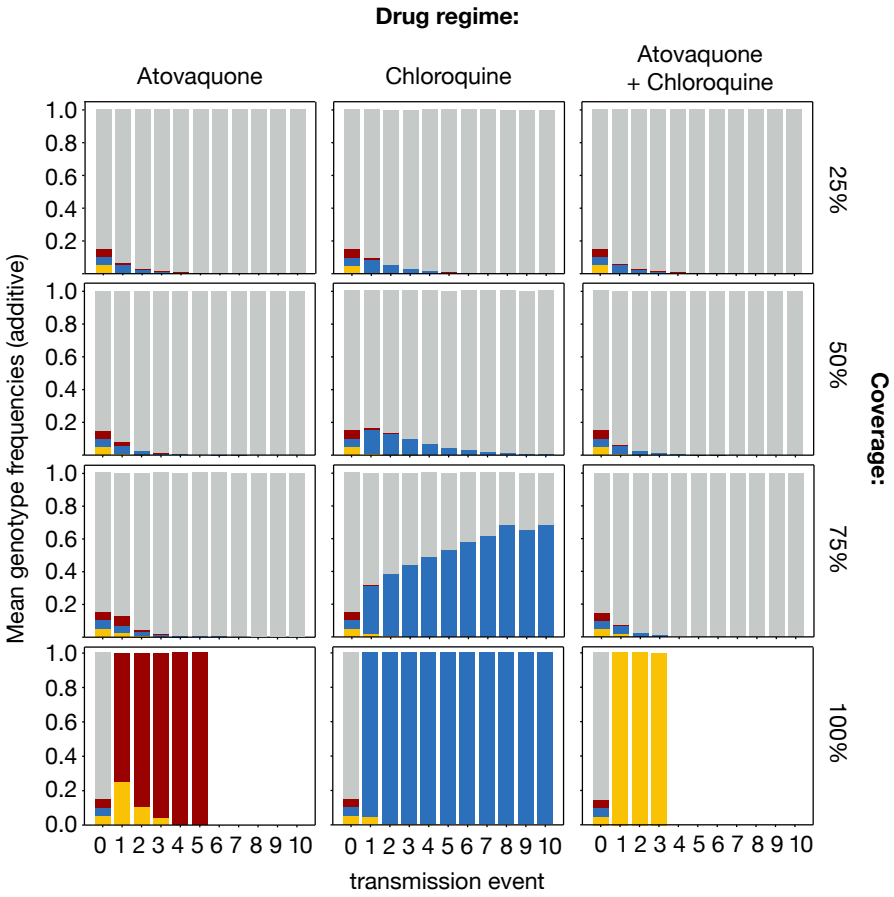


Figure 7.4: **Evolutionary dynamics of the parasite pool in different drug regimes and drug population coverages (mean of 100 realisations)**. Mean genotype frequencies for the parasite population at the end of each transmission event are shown as cumulative bars. All drug treatment scenarios start with the same initial condition, prevalent wild-type (grey) and equal proportions of single atovaquone resistant (red), single chloroquine resistant (blue), double resistant (yellow). Dynamics are shown for three drug regimes: atovaquone, chloroquine and both atovaquone and chloroquine; in low (25%), medium (50%), high (75%) and full (100%) population coverage. The sum of all genotype mean frequencies has been normalised to 1 for all transmission events in which parasites were present. Blank events correspond to the absence of parasites.

*Evolutionary dynamics: low levels of viability in mosquitoes in partial drug coverage prevents the spread of resistance*

Results show presence of the wild-type parasite strain after ten transmission events in all treatments for low (25%), medium (50%) and high (75%) coverage; however, full (100%) drug coverage provokes the resistant strains to outcompete the sensitive strains for all drug regimes (Fig. 7.4). When comparing atovaquone and chloroquine, we observe that the difference in the viability value (for atovaquone, 0.05; for chloroquine, 0.3) changes the frequency of resistant strains especially in high drug coverage. For low drug coverage, resistance does not spread in any of the single drug regimes. For medium drug coverage of chloroquine, resistant strains of chloroquine remain in low frequency until the 9th transmission event; in the 10th event only sensitive strains are present. In contrast, under medium coverage of atovaquone the resistant atovaquone strains remain only until the 3rd transmission event: the antagonistic selective pressure is strong enough to prevent their further spread. High drug coverage for chloroquine provokes maintenance of resistant strains at frequencies higher than 0.4 for all transmission events after the 3rd; whereas high atovaquone coverage prevents resistance spread from the 4th event. In the scenarios of full drug coverage, resistant strains take over the parasite population from the 1st event in all drug regimes.

The atovaquone-chloroquine drug combination shows qualitatively similar results to the atovaquone treatment, with an increased efficiency in interrupting parasite transmission in full drug coverage. For low, medium and high drug coverage, the resistant strains remain in very low frequencies, while the double-resistant takes over the parasite population after the 1st event when there is full coverage.

Overall, low levels of viability result in a stronger selective pressure than drug treatment under partial drug coverage, preventing the resistant strains to spread. Chloroquine resistance, which has more viability, can remain for longer in the parasite population than atovaquone resistance. In contrast, full drug coverage reverses the selection and resistant strains can outcompete their sensitive counterparts despite the mosquito disadvantage.

*Ecological dynamics: full drug coverage facilitates interruption of transmission of the parasite in single atovaquone and combined drug regimes*

To interpret the results from evolutionary dynamics, we need to understand the variations in the ecological dynamics. When studying the parasite population size, we observe that the exponential decay of the pool size increases in accordance to the drug coverage and that its slope value depends on the drug regime (Fig. 7.5).

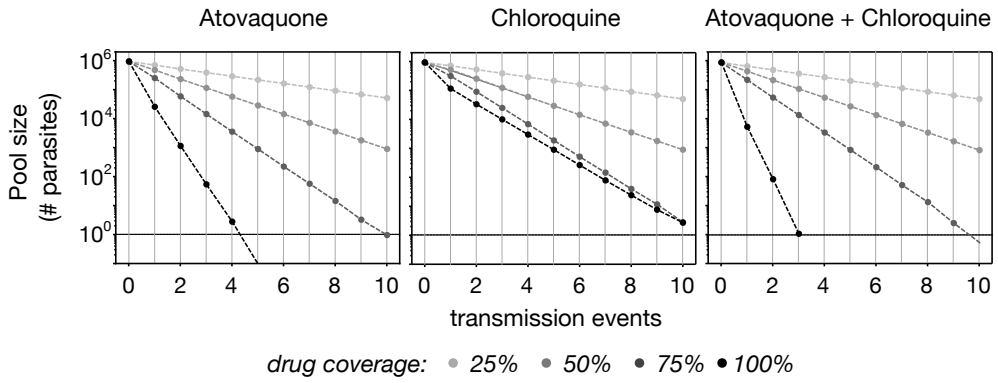


Figure 7.5: **Ecological dynamics of the parasite pool in different drug regimes and drug population coverages (mean of 100 realisations)**. Size of the pool (as mean number of parasites, in logarithmic scale) is updated after each of the ten transmission events, depending on the drug regime – atovaquone, chloroquine and both atovaquone and chloroquine – in low (25%), medium (50%), high (75%) and full (100%) population coverage (with grey scale correspondance). Population extinction is indicated in the event in which occurs as a white dot below the threshold of population with one parasite (line).

The rate of population decay for low and medium treatment is similar for all the single and combined drug regimes: by the end of the 10th event, population size is reduced to $< 10^5$ with low drug coverage and $< 10^3$ for medium drug coverage.

For high population coverage, the first transmission interruption occurs for atovaquone and the combined atovaquone-chloroquine regimes (before the 9th transmission event) (Fig. 7.6). For chloroquine, transmission interruption happening in the 10th transmission event is the most common scenario. However, full coverage reduces the population size rapidly (Fig. 7.5), interrupting transmission in the 4th event for single atovaquone and the 3rd event for combined atovaquone-chloroquine treatment (Fig. 7.6). For chloroquine, transmission interruptions occur similarly in high and full drug coverage, being the most frequent event of interruption in full coverage (Fig. 7.6).

In general, ecological dynamics show that there is little difference in the time of transmission interruption between the single atovaquone and the combined atovaquone-chloroquine treatment, especially when considering the variation caused by stochastic effects. Results are similar for the different drug regimes when the treatment is administrated to 75% of the population.

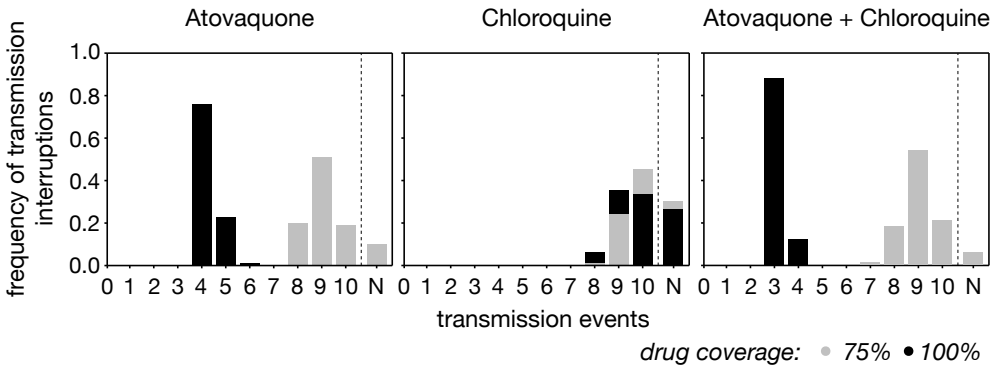


Figure 7.6: **Variance in the event of transmission interruption in different drug regimes and mass drug administration scenarios (variance in 100 realisations)**. In one life cycle extinction of the parasite can occur in the humans or in the mosquito. Transmission interruption occurs when during one event all the parasites are eradicated in all lifecycles. A 100 realisations of this process provide a distribution of the time when such transmission interruption occurs. Distribution of the number of realisations in which transmission interruption occurs in high (grey) and full (black) drug coverages. The event number corresponds to the event in which transmission interruption occurs (i.e. no parasites at the end of the event). Transmission interruption can occur later than the 10th transmission event (event N).

*Eco-evolutionary dynamics and drug efficiency: low drug coverage maintains the wild-type genotype causing drug treatment to be effective for longer*

The interaction between ecological and evolutionary dynamics needs to be analysed to understand the cause of parasite extinctions. Low drug coverage relates to high numbers of parasites, mostly drug-sensitive: this leads to high numbers of parasite population extinctions in the human host. Contrarily, full drug coverage makes the population of parasites decay rapidly, with only survival of resistant strains: although in the first event several extinctions in the human occur, further extinctions happen only in the mosquito because of the low viability of selected resistant parasites (Fig. 7.7).

For all drug regimes, low and medium drug coverage provoke more extinctions in the human host than in the vector, due to the high frequency of wild-type parasites. High population coverage with a single drug shows a similar number of extinctions in the two locations, with chloroquine regime showing more extinctions in the mosquito. Full coverage shows, for single drug regimes, more extinctions in the mosquito than in the human. When observing the double drug regime, instead, the number of extinctions in humans is greater than in mosquitos for all drug coverages (Fig. 7.7a).

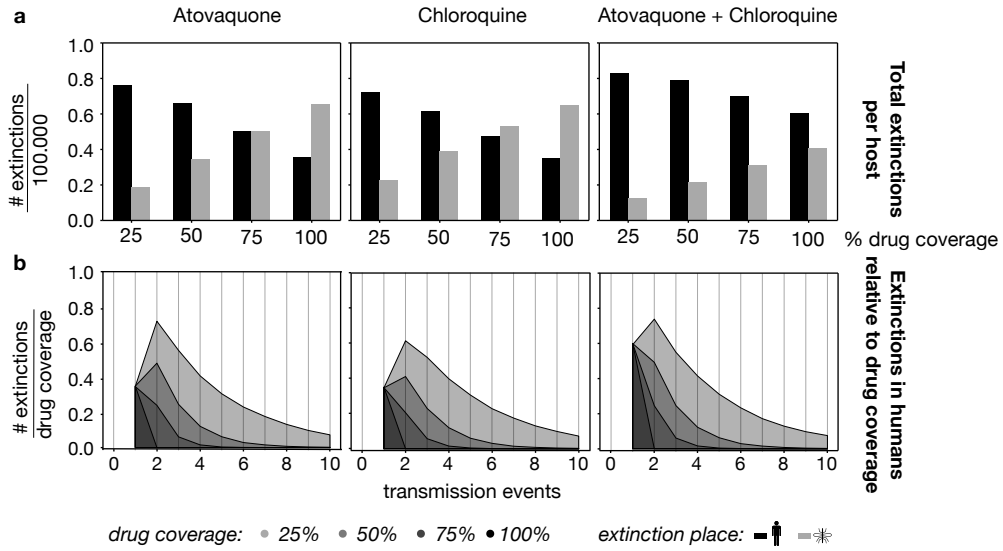


Figure 7.7: **Extinctions in the parasite population in different drug regimes and drug population coverages (mean of 100 realisations)**. a) Mean number of extinctions that occur in the host (black) and the vector (grey) per drug treatment during all the transmission events under different drug coverage values –low (25%), medium (50%), high (75%) and full (100%)– , in bars. b) Mean number of extinctions that occur in the human host, relative to the drug coverage (in grey scale), in each transmission event.

From a medical perspective, the goal of drug efficiency is to prevent infection in human individuals. A proxy for efficiency would be the number of parasite extinctions in human hosts along the ten transmission events (Fig. 7.7b). Because of the differences in drug coverage, we observe the extinctions relative to the population that receives prophylactic treatment. The results thus indicate the utility of drug treatment in each human population. Importantly, we do not compare the total number of extinctions in humans per transmission event, but we demonstrate the relationship between extinctions and the number of treatments, i.e. efficacy.

This results show that low population coverage is, for all drug regimes, the most efficient treatment along all the events. In the first event, all drug coverages have the same efficacy because of the initial genotype frequencies, and only differing between drug regimes. The double-drug regime has more efficacy because it can eliminate more parasites than the single-drug regimes.

However, along the transmission events, the genotype frequencies change according to the evolutionary dynamics and this affects the efficacy. Drug coverage scenarios that favour the prevalence of sensitive-strains show more efficacy. Besides, the ecological dynamics also influence the treatment efficacy: when the population size is big, more humans get infected and the treatment becomes useful for more in-

dividuals. Thus, despite the similar frequencies of sensitive strain that low and high coverage of atovaquone have, treatment loses efficacy in high coverage because the low number of existing parasites. For full coverage, the treatment is very efficient in the first event, but the spread of resistant strains stops the direct effect of human healing of the treatment. This phenomena is accentuated in the double-drug regime.

## 7.7 DISCUSSION AND CONCLUSION

The finding by Goodman et al. (Goodman et al., 2017; Goodman et al., 2016) that maternally inherited resistance alleles in malaria plasmodia to the drug atovaquone show limited viability in mosquitoes, led them to speculate that this could be exploited to limit the increase in frequency of such alleles in circumstances where the drug in question is widely employed, e.g. MDA. By developing and implementing our life cycle based model we have confirmed that drug resistance management is indeed feasible (Fig. 7.4), even in MDA programmes where $\gg$70% of the human population may be receiving the drug.

Given that globally, best practice requires the use of drug combinations for malaria treatment and prophylaxis, we chose to incorporate into our model a second drug. In doing so, we selected chloroquine, which in common with the vast majority of malaria drugs has resistance alleles encoded on the plasmodia nuclear chromosomes. Moreover, some disadvantage in the reproduction of chloroquine-resistant parasites has been reported in previous literature (Mharakurwa et al., 2011, 2013).

In our model, we studied the effect of antagonistic selection pressures by including fitness parameter estimates for atovaquone and chloroquine. However, any combination of drugs with resistance alleles with suitable patterns of inheritance could be modelled across a wide range of parameter values. It is essential to note that in our modelled scenarios, resistance alleles are already initially present. The rationale for not incorporating the emergence of resistance is appropriate as there is no evidence that MDA programs increase the probability of resistance alleles arising, beyond that resulting from other patterns of drug administration (Eisele, 2019; White, 2017).

The insights of our MDA model relate to the change in frequencies over time of drug-resistant genotypes and impact on the goal of timely interruption of malaria transmission in an isolated population. The evolutionary findings of our model confirm the intuitive and established Maude et al., 2009 result, that where resistance alleles are present in the parasite population, low (25%) and medium (50%) levels of population drug coverage should not act to substantially increase resistance fre-

quency. However, at higher coverage treatment regimes ≥75% the low viability of resistance alleles for one drug in the mosquito vector can usefully manage the rise of resistance alleles for another drug.

The comparison between single and double drug treatment shows little or no synergistic interaction in combined drug use, in terms of resistance allele management. Chloroquine nuclear-encoded resistance, given by PfCRT mutation K76T (viability = 0.3), increases both in 75% and 100% treatment coverage (Fig. 7.4) . The impact of atovaquone with the much more substantial reduction in viability (viability = 0.05) is different, as the frequency of resistance alleles only increases under full 100% coverage. The atovaquone treatment outcomes turn out similar to the combined atovaquone-chloroquine regime, suggesting a single drug with very low viability would be sufficient for MDA programmes. This result is observed even under our conservative (but untested) assumption that the mosquito viability disadvantage is multiplicative for the double resistant plasmodia individuals.

With respects to the ecological findings of the model, we are interested in the capacity to achieve local interruption of malaria transmission. Even starting from an unrealistically high sporozoite rate of 100% where all the female mosquitoes initially are infective (1 to 2 orders of magnitude higher than observed in field studies in high transmission areas), eliminating transmission is still possible where a large proportion of the human population consents to continuous drug treatment (Figs. 7.5 and 7.6). The transmission interruption occurs within a much smaller number of transmission events in the scenarios where a drug with a substantial reduction in resistant genotype mosquito viability is employed – i.e. more than two times faster for atovaquone than for chloroquine treatment. (Fig. 7.6). As with the evolutionary dynamics, there is little indication of synergy, with the combination drug treatment only marginally quicker than under atovaquone alone. This is even more noticeable when we consider the variance in the number of transmission events required for transmission interruption (Fig. 7.6).

The combined eco-evolutionary findings of the model provide insights into the trade-off between the speed with which transmission interruption can be achieved and the extent to which resistant genotypes, including double drug-resistant ones, rise in frequency. Were it feasible to treat 100% of the population with either atovaquone or a combination of drugs, resistant alleles would probably rise to high frequency (Fig. 7.4). This would not prevent the MDA driven decline in the number of plasmodia circulating in the pool (Fig. 7.5) nor the corresponding reduction in the number of cases of human malaria (Fig. 7.7). On the other hand, it would mean that medical interventions to treat patients would likely not be able to rely on the effectiveness of any of the classes of drugs employed in the MDA. Consequently, in

the attempt to eliminate transmission rapidly, it would be wise to make alternative drugs available to medical services that are likely to retain their effectiveness for case treatments. If a considerably slower path to transmission interruption is sought, with a population drug coverage of 75% (Figs. 7.5,7.6), high frequency of resistance can be avoided by using atovaquone or a combination of both drugs (Fig. 7.4). Further strategies could be investigated using the model, such as drug cycling or purposely changing drug population coverage during the treatment programme.

While our model can confirm many of the hoped-for predictions stemming from the observation of reduced viability of drug-resistant genotypes in the mosquito vector, we do not support a substantial degree of synergy stemming from a combination of resistance alleles that share this property. Consequently, it could be reasonable that an MDA based on a single drug with the most suitable properties may be sufficient to achieve malaria control goals. However, the history of malaria control indicates it is rarely wise to rely on a single mechanism, and other factors not included in our model may limit or enhance the importance of the factors highlighted here. For example, within-human fitness costs reported for resistant parasites (Huibjen et al., 2018) could intensify the plasmodia killing effect in humans beyond the substantial mosquito effect described here (Fig. 7.7a). Not explained in our model is the fact that chloroquine resistance alleles, despite inferred viability loss in mosquitoes, remain at appreciable frequencies around the world (Laufer et al., 2010). The model can thus be extended to capture more realistic scenarios, alternatively, it might be possible that genotype-specific estimates of viability in mosquitoes are to some degree context-dependent. Scenarios include the possibility that the estimated viability disadvantages of resistance alleles may be smaller than estimated (Blake et al., 2017) or may even be selected to decrease during a long-term MDA effort.

The model, as described in this paper, follows each step of the life cycle of the *Plasmodium* using available parameter estimates (Rosenberg, 2008). We envision that the model will provide a convenient basis for further elaborations. Complex models (e.g. including epidemiology, spatio-temporal heterogeneity in drug coverage) would then move towards informing the role MDA could play in leveraging genotypic viability differences towards the goal of eliminating malaria within a generation.

## SUPPORTING INFORMATION

*Assessing the levels of viability in chloroquine-resistant parasites*

Chloroquine resistance is well documented, as it emerged in the late 1950's in Asia and nowadays it affects several countries with endemic malaria. Thus, although a disadvantage has been reported for chloroquine-resistant parasites in the mosquito phase, it has not prevented the spread of resistance. Here we test different levels of viability for the parasite genotypes with the resistance mutation, to be compared in the main results with the atovaquone-resistant parasites (with a very low viability of 0.05).

The values tested in this section are the following: 0.05, 0.11, 0.3, 0.5, 1.0 (Fig. 7.8). The parameter range of viability is from 0 to 1; thus, in the lower range we choose 0.05 as equivalent to the atovaquone-resistance viability and we choose the upper extreme 1 in which there is no disadvantage. Because the paper (Mharakurwa et al., 2013) reports a 9-fold disadvantage of the resistant parasites, the value 0.11 is also tested. In the intermediate range, 0.3 and 0.5 are chosen arbitrarily.

Given the known spread of chloroquine resistance, the final choice corresponds to the smallest value which shows resistance maintenance in medium and high levels of drug coverage. The lower values 0.05 and 0.11 underestimate the viability of resistant zygotes: even in scenarios of high drug coverage (75%), resistance does not show to be prevalent from early transmission events. Disregarding the full viability of maximum value 1, which quickly allows the resistant parasites to reach high genotype frequencies, we can focus on the intermediate values: 0.3 and 0.5. Given that the prevalence of resistant chloroquine strains is usually less than 20% in countries in which drug coverage is not massive (i.e. scenarios of low or medium drug coverage), both could be realistic. However, for the focus of the study and the comparison with atovaquone-resistant parasites, we prefer not to overestimate the viability and thus choose as adequate value of study a viability of 0.3.

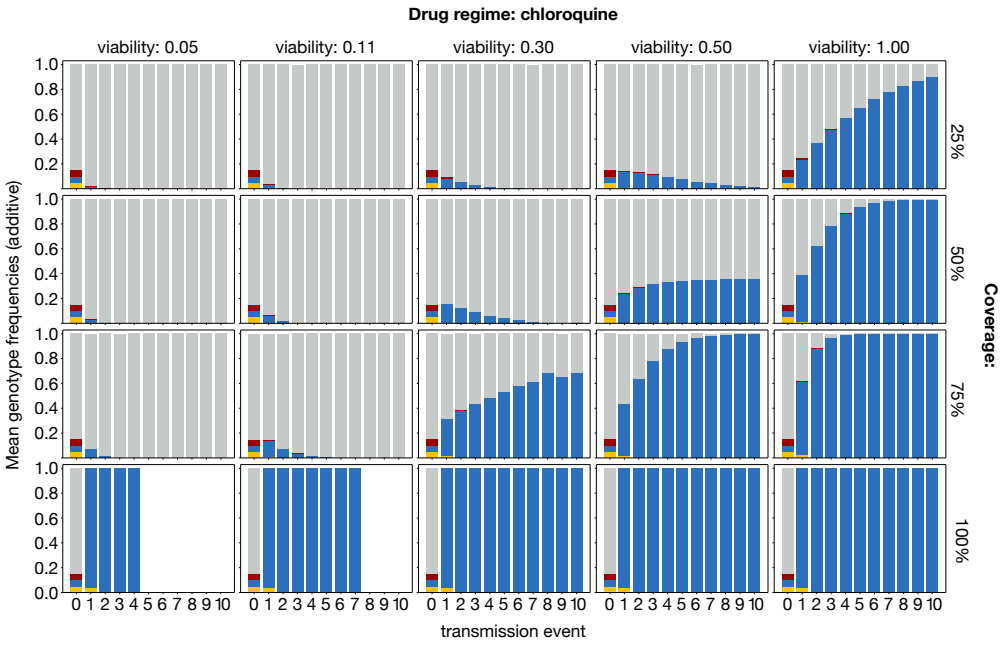


Figure 7.8: **Evolutionary dynamics of parasite pool in different values of viability of chloroquine-resistant parasites (mean of 100 realisations)**. Mean genotype frequencies for the parasite population at the beginning of each event are shown as cumulative bars. All plots correspond to chloroquine treatment scenarios, with low (25%), medium (50%), high (75%) and full (100%) population coverage. All plots start with the initial frequencies for wild-type (grey), single atovaquone resistant (red), single chloroquine resistant (blue), double resistant (yellow). The sum of all genotype mean frequencies has been normalised to 1 for all transmission events in which parasites were present. Blank events correspond to transmission interruption.

# 8

# PERSPECTIVES

In the research project presented in this part, I have modelled the life cycle of *Plasmodium* and used it to study disease transmission under mass drug administration treatment. I consider the computational implementation of the life cycle, at the local scale, the core part of the model; useful for further malaria studies with different questions due to its detailed representation of the biology and its correspondence to experimental data from Rosenberg (2008). Nonetheless, the global scale of our model is also useful to study other scenarios, changing initial parameter values or exploring some of the assumptions made. Here I describe model extensions for different initial conditions, changes in the modelling of the drug effect and treatment, and changes in the modelling of disease transmission.

## 8.1 MODEL EXTENSIONS

*Exploring a diversity of initial scenarios*

In the research presented, we constrain the study of eco-evolutionary dynamics to a naive population of 100.000 humans each of which is bitten at the beginning of a transmission event. The model assumes that all mosquitoes are infectious; i.e. that each bite transmits sporozoites and infects the human host. In real scenarios, only a fraction of the mosquitoes transmit the disease, so variations in a range of 0 to 100% of infectious mosquitoes – formally, the sporozoite rate (Birley and Charlewood, 1987) – could be explored. Expectations are this would slow down the dynamics, but maintain the outcome of each strategy. Drift could affect the outcome at very low infectivity values.

Also, in all scenarios explored the initial genotype frequencies of the parasite are the same: only a 15% of the initial parasites have alleles for drug resistance, with a frequency of 0.1 for each resistant allele $A$ and $C$. Similarly to the previous proposal, we could explore a range of values for initial allelic frequencies and analyse the outcomes. We would expect relevant variations in the results. Thus, it would be important to observe at which frequency values there is a change, for a better assessment of MDA treatment in regions with higher prevalence of resistant alleles.

These proposals correspond to an extended computational analysis, which does not change the model itself.

*Remodelling the drug effect*

In the current version of the model, the drug effect is modelled within the equation for the growth rate of the parasite (Eq. 8.1):

$$r_i(j,k) \quad = \quad b_j - (1 - w_i(j,k))2b_j. \tag{8.1}$$

When the parasite is susceptible and the drug is applied ($w_i(j,k)$=0), it dies at a rate equivalent to its growth ($r_i(j,k) = -b_j$). This symmetry is an artificial simplification of the drug effect, which is useful to predict the outcome assuming full drug efficacy (i.e. all susceptible parasites die) and allows variation in parasite resistance. We could, however, model the drug effect considering two more ideas: mode of action and dosage.

Both atovaquone and chloroquine can kill the parasites and prevent their further development. Thus, it is appropriate that their presence induces a negative growth rate for the parasite ($r_i(j,k) < 0$); however, some drugs, because of their mode of action, can prevent reproduction but they do not kill the existing parasites. Additionally, the drug concentration available determines the level of growth inhibition and toxicity: low drug dosage reduces the treatment efficiency and the current model cannot represent that aspect.

Both ideas could be implemented by changing the constant term $2b_j$. To inhibit growth, it would be replaced by $1b_j$, so the susceptible parasite growth rate is null when treated ($r_i(j,k)$=0). To include drug dosage we could, for example, substitute the term for a saturating function: at more drug, more toxic effect, up to a limit. The most adequate function would depend on the drug pharmacodynamics.

## 8.2 DRUG INTERACTIONS AT THE INDIVIDUAL AND POPULATION SCALES

*A problem with the current combined drug regime*

When designing the study, we decided to theoretically explore the scenario in which both drugs – atovaquone and chloroquine – were given in combination. We expected a quicker transmission interruption of the disease at the population level because the double-resistant parasites had an increased disadvantage in viability. Results did not differ much than with atovaquone alone, so the single-atovaquone regime would be preferred, to avoid unexpected side effects or minimise costs. However, we did not take into account a key factor in combined drug treatments: within patient drug interactions.

Previous studies have reported antagonistic interaction between chloroquine and atovaquone (Canfield et al., 1995; Co et al., 2009). Because of their mechanism of action, they interfere with each other and the half maximal inhibitory concentration, $IC_{50}$, is higher when they are given in combination; i.e. more drug is needed to obtain the same effect. Thus, it is not convenient to administrate them in combination, independently of the population-level results.

Nonetheless, combined drug treatments within the patient are not the only strategy to include multiple drug selections for the parasite: treatments can be mixed within the population, or cycled along time.

*Alternatives: drug mixing and sequential treatment*

**Drug mixing.** Combined treatments act within one host individual, and each parasite receives simultaneously two drug selection pressures. In drug mixing, different individuals are treated with different drugs: each host imposes single-drug selective pressure to each parasite, but selection is heterogeneous at the population level (Raymond, 2019). This avoids drug interactions within the host, interferences in toxicity and increased side effects. Drug mixing could be implemented in our model by treating a proportion of the human population with atovaquone and the rest with chloroquine.

**Drug cycling and sequential therapy.** Drug selection heterogeneity could also be distributed temporally. Currently, the parasite undergoes different selection pressures during the life cycle due to the host-vector rotation. Cycling the drug treatment would add another layer of temporal heterogeneity. The cycling of drugs could be done at two levels: at the population level – as commonly seen in hospitals–, or at the individual level, with a sequential treatment for each patient (Roemhild and Schulenburg, 2019). Cycling at the population level could be implemented by alternating the drugs at each transmission event, while sequential treatment would require to define a length in days from which to change the selection within the local life-cycle level.

## 8.3 RE-MODELLING DISEASE TRANSMISSION

*Entomological inoculation rates and overlapping generations*

A frequent concept used in modelling of malaria transmission is the entomological inoculation rate (EIR), which has been dismissed in our model. The EIR estimates the number of bites by infectious mosquitoes per person per unit time and it is used as measure of the intensity of transmission (McDonald, 1957).

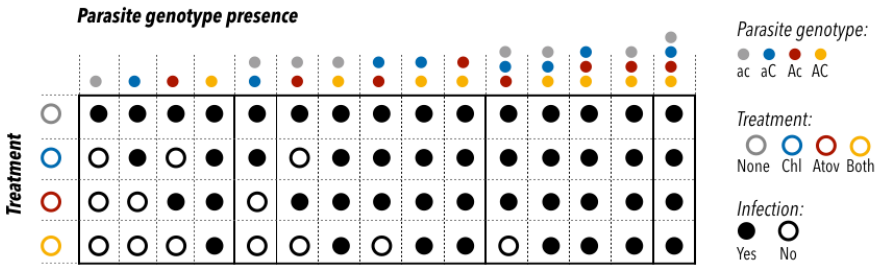


Figure 8.1: **Treatment efficacy in presence of single or multiple parasite genotypes.** When the mosquito bites the human host, it can infect with a single or multiple strains of the parasite. The table shows the treatment efficacy, i.e. if the infection occurs (black) or not (white), for each treatment and possible combination of parasite genotypes within a human.

In our model, the EIR would be 1 per transmission event (being the duration of a transmission event 36 days). Because MDA is only recommended in areas of low transmission, this could be plausible. However, the EIR is higher for many countries with endemic malaria: in some African regions the annual EIR is higher than 500 (Kelly-Hope and McKenzie, 2009).

Increasing the EIR would require including overlapping generations of parasites within the human host, as humans would get more than one mosquito bite within a transmission event. Apart from increasing the parasite load, this would affect the treatment efficacy: multiple bites increase the probability of getting infected with a double-resistant parasite. Despite being in low frequency, the double-resistant parasite survives to all treatments and allows the parasite to reproduce within the human host (Fig. 8.1).

Finally, there are models which relate an increased EIR with lower spread of resistance (Legros and Bonhoeffer, 2016). The reason behind is within-host competition of susceptible and resistant parasite strains. If drug resistant parasites carry a fitness cost, in absence of treatment the susceptible parasites reproduce and spread faster. When a human individual is bitten more than once, it increases the probability to get susceptible parasites (or their initial frequency), which in absence of treatment, increase their frequency within the human host. In our model, we didn't include cost of resistance in the human because the mutants already have a cost of resistance in the vector stages, but it could be included.

In conclusion, the model can be improved by, at least, changing the combined treatment for a drug mixing strategy, but also adding the possibility for increased disease transmission, to make it more applicable and flexible to different geographical areas affected by malaria.

Part IV

# DISCUSSION

# 9

# GENERAL DISCUSSION

During this thesis, I presented scenarios in which a temporal variation of selection allowed the reduction of pathogen populations, with a focus on the alternation of their host organisms. In these scenarios, an implicit human agent aimed to control the disease caused by the pathogen; thus, each possibility for selection alternation was regarded as a potential strategy.

The thesis, as stated in the introduction, aimed to assess the optimality of these disease management strategies. I conclude here that, with the design of theoretical models, I have been able to judge the outcomes of the studied strategies with an eco-evolutionary perspective. The following is a compilation of the current findings. Because the optimal strategies or patterns of alternation are context-dependent, any further assessment needs new research.

## 9.1 SUMMARY OF RESEARCH FINDINGS

*Plant disease control through rotations of crop types*

In agriculture, rotations of crop types and alternations of disease management strategies in a field can slow down the growth of a pathogen and control the infection build-up along harvesting seasons. At its turn, this results in lesser yield loss and general improvement of the field performance.

**Research.** In the first project, I studied a generic plant disease when crops are rotated in a single field. From the farmers' perspective, it was known that crop rotations are beneficial for both improving soil quality and controlling pests along harvesting seasons. From the evolutionary perspective, it was known that switching the environment in which a pathogen grows can limit its reproduction and change its evolution. However, the coupling of the two concepts in a formal model was missing. Thus, I used knowledge from evolutionary theory to integrate pest control in a model using agronomic criteria to assess crop rotations. Given a field where cash crops and cover crops can be alternated, I asked which rotation patterns maximise yield under an infection which affects cash crops, but not cover crops. The model showed that the long-term yield outcome of a rotation pattern depends on its ability to both maintain soil quality and diminish pathogen load during the seasons.

**Assessment.** Regular rotations that switch every other year may not perform the best, due to characteristics of how soil quality and the pest build-up. Instead, investing first in soil quality can maximise the yield in the first cash crop seasons, in which the infection is not severe. When consecutive cash crops are cultivated, further alternation with cover crops is needed to face both pathogen load and soil quality depletion. Pathogen evolution needs to be taken into account, as the pathogen load of virulent strains increases faster and provokes more yield loss.

**Discussion.** The model presented is generic, meaning it does not apply to a particular species of crop and pathogen. In consequence, results cannot be translated directly to the field. Nonetheless, this feature is advantageous for future applications: the model is a tool that can adapt to several species of interest, especially to those crops affected by soilborne pathogens, and assess the performance of a specific rotation.

*Plant disease control through the integration of management strategies*

As continuation of the first research project, I applied the crop rotations model to a specific crop disease and adapted the modelling features to the life-cycle of the pathogen. I included two other pest control strategies: application of fungicides and the use of crop variants with genetic resistance, and studied the three methods in combination.

**Research.** I showed the applicability of the generic model to the study of *Sclerotinia sclerotiorum* in oilseed rape. *Sclerotinia* is a fungus known to be controlled by crop rotations due to its life cycle: to survive and reproduce, it relies in sclerotia (soil structures), which remain in the soil from season to season. When a non-host crop is cultivated, sclerotia germination is ineffective and the soil reservoir declines. The increased cropping frequency of oilseed rape in the last decade aggraviated epidemics of *Sclerotinia* and short rotations or fungicides alone are often not cost-effective. In my model, I explored the outcomes of integrating rotations of oilseed rape with break crops, fungicide application and cultivars with genetic resistance, by simulating host-pathogen interaction dynamics. I studied different lengths of break crop seasons, and different levels of fungicide efficiency and genetic resistance. Results showed that the combination of low resistance and low fungicide with rotations could control the build-up of the infection.

**Assessment.** Farmers are interested in using short rotations of oilseed rape, but they cannot control the build-up of the infection. Given that, the integration of rotations with fungicides and/or genetic resistant variants would be recommended, as low efficacy of single control methods can be synergic when combined. This can

lead to an efficient and durable control, sustainable across generations. However, the cost-effectiveness of the methods is not contemplated, and this is necessary to determine its field applicability.

**Discussion.** A parameter optimisation is required to adjust the model dynamics to field data and increase the reliability of the results. Also, pathogen evolution to overcome the stressors – i.e. fungicides and crop host genetic resistance – needs to be taken into account. As mentioned, a proper assessment needs to consider the economic and environmental costs of the strategies before they are put in practice. Overall, the application of the generic model opens doors to new research questions to study.

*Vector-borne disease control through drug-induced antagonistic selection*

In vector-borne diseases, the vector organism is a mechanical mean of transmission, but also exerts an evolutionary pressure to the pathogen. Knowing the differences between the selective forces of the two ecological niches is necessary to predict the outcome of pathogen eradication strategies and pathogen resistance management.

**Research.** I investigated the consequences of antagonistic selection between the human host and the mosquito vector for *Plasmodium* strains with resistance to antimalarials. Previously, experimental researchers showed that atovaquone-resistant parasites have low viability in the mosquito stages of the reproductive life-cycle. Also, mass drug administration (MDA) is currently regarded for malaria control, but concerns of its selection for resistance parasites prevent its application. In the model, I explored different levels of population drug coverage, under which the antagonism between selective pressures could potentially prevent the spread of antimalarial resistance. I studied the dynamics of parasite genotype frequencies along events of disease transmission between populations, as well as the extinction of the parasite population. Results showed that low population coverage of antimalarials prevents resistance spread, whereas mass drug administration facilitates the interruption of disease transmission.

**Assessment.** There is a trade-off between resistance spread and transmission interruption, which can affect the interpretation of drug efficacy. When a small fraction of the population receives the drug, the drug is effective for the treated individuals, as the parasite is susceptible. When a huge fraction of the population receives the drug – i.e. MDA –, the drug is ineffective for treated individuals after very few generations, as the parasite is resistant. However, MDA provokes a rapid decline of the total parasite population, which allows for transmission interruption, so parasite

eradication, which is more effective in the long term.

**Discussion.** The study is very limited for its assumptions; isolated population without parasite migration, transmission between naive human populations, and non-overlapping generations of parasites inside a human (one bite per human). The population scale of the model should be modified using further epidemiological features to be more realistic. However, studying the eco-evolutionary dynamics at the life-cycle scale can be useful, and the global results present the antagonism of selection pressures in atovaquone resistant parasites as a promising mechanism for malaria eradication strategies.

## 9.2 APPLICABILITY OF THE FINDINGS

The studies presented aim to provide knowledge that can be applied, assessing the outcome of different strategies. The crop disease models are an example of how eco-evolutionary theory can complement farmers' knowledge. On the other hand, the vector-borne disease model emphasises the role of the vector as a selective force, and in consequence, the need for evolutionary theory for improving human health. However, the bridge between the findings and their actual application in management practices is long. Until this point, I have discussed the limitations and assumptions underlying the models, mostly from the eco-evolutionary perspective. Here I present additional evaluation steps which would need to be regarded when considering the application of the studied strategies.

### *Differences in the criteria for cost-benefit balance*

Scientists and practitioners can have different views on the balance of costs and benefits of management strategies. In the case of agroecosystems, scientists focus on the processes that involve the species, while farmers, or other practitioners, tend to focus on the agroecosystem outcomes – i.e. yield and profits. This leads to a mismatch between research results and their applicability, as pointed by Kleijn et al. (2019). For example, farmers assess the market value of the crop product (benefit), together with the economic cost of spraying fungicides (cost). Fundamental science, instead, tends to assess the density of healthy plants (benefit) and the effects of fungicides for other species in the agroecosystem (cost).

We need to learn to find common interests to approach the two perspectives. In the example, the more healthy plants, the more crop product to be sold in the market. And minimising the use of fungicides reduces both its costs of application and its environmental impact. Also, we need to learn which practices are preferred

by the farmers. For example, farmers generally like the use of crop rotations, but many of them dislike practices such as beetle banks or wildflower strips; which might be perceived as a loss of land or complex mid-field modifications despite the evidence that they increase agronomic benefits. (Bailey, 2015; Kleijn et al., 2019; Pywell et al., 2015).

*Risk assessment at the individual and population levels*

The interplay between the individual and the population is crucial for risk assessment. In the crop fields, we assess the practices at the plant population level: we do not count individual plants, but rather measure plant densities. Overall, we seek for increased yield, which is a population-level measure. Instead, in human health, each person weights for risk assessment. The minimisation of the disease becomes more critical than the maximisation of health. The cost of failure, then, is perceived differently. When comparing our two general scenarios – agriculture and human health –, a risk for a farmer of losing 25% of its crops could be acceptable, but a risk of death for malaria of 25% of a local human population must be avoided. That prevents the application of strategies such as low population drug coverage, which could protect efficiently a minority (avoiding the spread of resistance) but it would expose the rest of the population to potential damage.

*Feasibility*

The scale at which the management practices work also affects their applicability. Questions such as how many people have to apply it, or how many people it affects determine if it is feasible or not to put in practice a particular strategy. For example, farmers can decide to work on the local scale of their farm, within governmental or institutional regulations. If they have enough resources available, they can apply a strategy at their own cost. In contrast, the mass drug administration proposed for the control of malaria only works if there is coordination between the government, health services and people. It involves the financial resources to afford drug treatments, and clinical administration must reach all the targeted population – which, at its turn, needs to accept the treatment. The evaluation of the success of previous practices which are similar is required for determining feasibility.

*Balance between short and long-term consequences*

In previous discussions, I have mentioned the difference between short-term and long-term outcomes. Evaluating the balance between the two time-scales is also crucial for the application of our findings. The same period might be regarded as

both short and long term, depending on the perspective. For a local farmer, the yearly outcome might be regarded as short-term, while a 10-years period would be long-term. For the cultivated land, however, a 10-years period would be short-term, if compared to the field use across generations of farmers. Likewise, the government of a country with endemic malaria can consider 5-10 years as long term, while programs for the global eradication of malaria can have different time criteria. How to assess the correct timeframe is very context-dependent, but in general, the sustainability of practices in the long-term should be taken into account. The consequences of the contrary are reflected, for example, in the current antibiotic resistance crisis.

## 9.3 FINAL REMARK: ECO-EVOLUTIONARY PRACTICES IN THE ANTHROPOCENE

Back in 2000, Paul Crutzen claimed that we were living in the "Anthropocene". The reason beyond this new term was the influence of the human species in shaping the current Earth geological time (Crutzen and Stoermer, 2000). Since then, the idea of an Anthropocene geological era gained popularity, especially with the threat of climate change and the subsequent need for development of sustainable practices. Understanding how the human action impacts the surrounding environment is needed, then, for both avoiding harmful practices and promoting beneficial ones. For that, the study of ecological and evolutionary principles is required; and must be applied to global challenges such as the design of efficient and sustainable food supply, improved human health, or conservation of biodiversity.

Approaches such as the ones presented in this thesis pursue an improvement of current practices applying these ecological and evolutionary points of view. I hope that the coming decade brings further contributions and science can be recognised, by everyone, as a central pillar in the resolution of the current societal and environmental issues.

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# LIST OF PAPERS, MANUSCRIPTS AND CONTRIBUTIONS

## LIST OF PAPERS AND MANUSCRIPTS

*Chapter 3:*
**Bargués-Ribera M** and Gokhale CS (2020). Eco-evolutionary agriculture: Host-pathogen dynamics in crop rotations. *PLoS Computational Biology* 16(1): e1007546. https://doi.org/10.1371/journal.pcbi.1007546

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## AUTHOR CONTRIBUTIONS

*Chapter 3:*
**MBR** and CSG conceived the study. **MBR** implemented the model and performed the analysis. **MBR** and CSG interpreted the results. **MBR** and CSG wrote the paper.

*Chapter 4:*
**MBR**, RGR and CSG conceived the study. **MBR** implemented the model and performed the analysis. **MBR**, RGR and CSG interpreted the results. **MBR**, RGR and CSG wrote the paper.

*Chapter 7:*
**MBR**, JH, NH, AM and CSG conceived the study. **MBR** implemented the model and performed the analysis. **MBR**, JH, NH, AM and CSG interpreted the results. **MBR** wrote the thesis chapter.

Chapters 1, 2, 5, 6, 8 and 9 are my own contribution.

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# DECLARATION

I hereby declare that:

i. Apart from my supervisor's guidance, the content and design of this thesis is the product of my own work. The co-authors' contributions are listed in the dedicated section;

ii. This thesis has not been already submitted either partially or wholly as part of a doctoral degree to another examination body, and no other materials are published or submitted for publication than indicated in the thesis;

iii. The preparation of the thesis has been subjected to the Rules of Good Scientific Practice of the German Research Foundation;

iv. Prior to this thesis, I have not attempted and failed to obtain a doctoral degree.

*Plön, January 2020*

Maria Bargués i Ribera

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