

THE ECOLOGY AND EVOLUTION OF PATHOGENS IN A CHANGING WORLD

Ian Forrester Miller

A DISSERTATION

PRESENTED TO THE FACULTY

OF PRINCETON UNIVERSITY

IN CANDIDACY FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

RECOMMENDED FOR ACCEPTANCE BY

THE DEPARTMENT OF

ECOLOGY AND EVOLUTIONARY BIOLOGY

Adviser: C. Jessica E. Metcalf

May 2022

Copyright by Ian Forrester Miller, 2022.

All rights reserved.

Abstract

Infectious diseases are an integral part of natural ecosystems but also pose a serious threat to human health and agriculture. Understanding the multitude of factors shaping the epidemiology and evolution of pathogens is crucial for predicting and mitigating their effects, especially in a rapidly changing world. In this dissertation, I present four studies about how vaccination, emerging pathogens, and climate change affect the epidemiology and evolution of infectious disease. In the first chapter I consider the effects of toxin targeting vaccines, such as acellular pertussis vaccines, on the evolution of pathogen virulence. In the second chapter I map projections of the expected burden of the novel coronavirus SARS-CoV-2. In the third chapter I assess the risk for COVID-19 vaccines to drive the evolution of increased viral virulence. In the fourth chapter I investigate how climate change will alter the epidemiology of fungal plant pathogens using the flax rust system as a model.

Abstract.....	3
Introduction	8
Overview.....	8
References.....	10
Chapter I: Vaccine-driven virulence evolution: consequences of unbalanced reductions in mortality and transmission and implications for pertussis vaccines	12
Foreword.....	12
Abstract	13
Introduction.....	14
Methods.....	20
<i>Virulence and immunity.....</i>	<i>21</i>
<i>Host population immunity structures</i>	<i>23</i>
<i>Epidemiological model.....</i>	<i>24</i>
<i>Evolutionary dynamics</i>	<i>26</i>
<i>Epidemiological consequences.....</i>	<i>29</i>
<i>Computational methods.....</i>	<i>30</i>
Code accessibility	30
Results.....	31
<i>Evolutionary outcomes</i>	<i>31</i>
<i>Epidemiological outcomes.....</i>	<i>34</i>
Discussion	37
Supplementary figures	42
References.....	50
Chapter II: Disease and healthcare burden of COVID-19 in the United States	56

Foreword.....	56
Abstract.....	57
Introduction.....	58
Results.....	62
Discussion.....	70
Extended data figures.....	73
Methods.....	83
<i>Data</i>	83
<i>Mechanistic models</i>	83
<i>Rescaling contact matrix</i>	85
<i>Model simulation</i>	86
<i>Case estimation</i>	87
<i>Case distribution</i>	87
<i>'Alternate optimistic' and 'alternate pessimistic' scenarios</i>	88
<i>Code availability</i>	89
References.....	89
Chapter III: Assessing the risk of vaccine-driven virulence evolution in SARS-CoV-2	92
Foreword.....	92
Abstract.....	93
Introduction.....	94
The theory of virulence evolution.....	95
Evaluating potential for virulence evolution in SARS-CoV-2	98
COVID-19 vaccines and virulence evolution.....	101
Bounding the effects of COVID-19 vaccines on selection for increased virulence	104

Discussion	111
Methods.....	116
<i>Epidemiological model</i>	116
<i>Evolutionary analysis</i>	119
<i>Code availability</i>	124
Supplementary figures	125
References.....	130
 Chapter IV: Predicting the effects of climate change on the cross-scale epidemiological dynamics of a fungal plant pathogen	 140
Foreword.....	140
Abstract.....	141
Introduction.....	142
Results.....	143
<i>Study system</i>	143
<i>Within-host processes</i>	146
<i>Transmission</i>	153
<i>Epidemiological model</i>	156
Discussion	158
Methods.....	161
<i>Data collection</i>	161
<i>Statistical Analyses</i>	168
<i>Epidemiological model</i>	175
Acknowledgements.....	181
Extended Data Figures	182
Supplementary information	192

<i>Supplementary Text 1: Environmental condition monitoring equipment.....</i>	<i>192</i>
<i>Supplementary Text 2: Absolute humidity calculation.....</i>	<i>192</i>
<i>Supplementary Text 3: Calculating values of X and Y in the tilted gaussian plume model</i>	<i>193</i>
<i>Supplementary Text 4: Plant location alignment</i>	<i>196</i>
Supplementary figures	197
References	204
Conclusion	209
Overview.....	209
Vaccines.....	209
Plant pathology	211
References.....	213
Acknowledgements	215

Introduction

Overview

A fundamental aim of ecology and evolutionary biology is to understand continuities and changes in the abundance and distribution of species and their genotypes, the structure of communities, and the stability of ecosystems. The spread of infectious disease is a factor broadly relevant to all these processes. Infectious disease results from the interaction between a ‘host’ organism and a ‘pathogen’ organism. Pathogens reduce the fitness of hosts by increasing their mortality or decreasing their fecundity.

The ubiquity of pathogenic interactions reflects their fundamental role in ecology; 30%-71% of all species have been estimated to be pathogens [1], and virtually all are parasitized. Indeed, pathogens have been found to regulate host population size [2] and constrain geographical host range [3,4]. They also are omnipresent in communities, affecting organisms in all trophic levels, from apex predators [5] to primary producers [6]. Including host-pathogen interactions in species interaction networks increases the stability of communities [7-9].

Studying the ecology, epidemiology, and evolution of pathogens through the lenses of ecology and evolution is important not only for advancing scientific knowledge, but also for controlling threats posed to society by infectious disease. Pathogens can have direct effects on societal wellbeing by causing disease in humans and agricultural systems, and indirect effects by disrupting ecosystems that provide essential services. In order to predict, mitigate, and engineer protection against these threats it is necessary to adopt mechanistic or semi-mechanistic analytical frameworks (e.g. compartmental epidemiological models) that capture the relevant

details of the system in question over ecological or evolutionary timescales [10]. A key strength of this methodological approach is that it facilitates prediction about the future state of infectious disease systems in addition to explaining drivers of observed patterns.

This future-facing approach to studying infectious disease is essential in a changing world. Technological advances, globalization, climate change, demographic shifts, emerging pathogens, and many other factors will alter current threats and introduce new challenges. In this dissertation, I present four studies about the epidemiology and evolution of infectious disease in a changing world.

In the first chapter, I investigate how toxin-targeting vaccines, which have differential impacts on transmission and mortality, might affect the evolution of pathogen virulence, or lethality. This question is particularly relevant for pertussis, a disease caused by the bacterium *Bordetella pertussis*, as modern vaccines target only the *B. pertussis* toxin and a small number of other proteins. I find that although evolutionary and epidemiological outcomes are worse when vaccines reduce mortality to a greater extent than transmission, variability in the efficacy of vaccinal immunity among individuals can limit negative outcomes. In the second chapter, I project the burden of the novel coronavirus SARS-CoV-2 in the United States, mapping numbers of hospitalizations and ICU admissions relative to population and healthcare system capacity. In the early stages of the COVID-19 pandemic, these results revealed that the burden of disease would not be limited to urban areas and could be highest in away from major population centers. In the third chapter, I assess the potential for SARS-CoV-2 vaccines to drive the evolution of increased virulence. While I conclude that the potential for this event is low, my findings emphasize the importance of ensuring lasting protection against infection, potentially through the use of booster doses. In the fourth chapter, I investigate how climate change might affect the

within and between host spread of fungal plant pathogens using the flax rust model system. My results indicate that while climate change may slow the spread of disease in the short term, rebounds in infection intensity and prevalence may occur over time due to continued warming.

References

1. De Meeûs, T., and F. Renaud. 2002. Parasites within the new phylogeny of eukaryotes. *Trends Parasitol.* 18:247–250.
2. Dobson, A. 1995. The Ecology and Epidemiology of Rhinderpest Virus in Serengeti and Ngorongoro Conservation Area. P. *in* Serengeti II: Dynamics, Management, and Conservation of an Ecosystem.
3. Antonovics, J. 2009. The effect of sterilizing diseases on host abundance and distribution along environmental gradients. *Proc. R. Soc. B Biol. Sci.* 276:1443–1448.
4. Bruns, E. L., J. Antonovics, and M. Hood. 2019. Is there a disease-free halo at species range limits? The codistribution of anther-smut disease and its host species. *J. Ecol.* 107:1–11.
5. Roelke-Parker, M. E., L. Munson, C. Packer, R. Kock, S. Cleaveland, M. Carpenter, S. J. O'Brien, A. Pospichil, R. Hofmann-Lehmann, H. Lutz, G. L. M. Mwamengele, M. N. Mgasa, G. A. Machange, B. A. Summers, M. J. G. Appel, R. Hofmann-Lehmann, S. J. O'Brien, and S. Cleaveland. 1996. A canine distemper virus epidemic in Serengeti lions (*Panthera leo*). *Nature* 379:441–445.
6. Lawrence, G. J., P. N. Dodds, and J. G. Ellis. 2007. Rust of flax and linseed caused by *Melampsora lini*. *Mol. Plant Pathol.* 8:349–364.
7. May, R. M. 1972. Will a Large Complex System be Stable? *Nature* 238:413–414.
8. Allesina, S., and M. Pascual. 2008. Network structure, predator - Prey modules, and

- stability in large food webs. *Theor. Ecol.* 1:55–64.
9. Dobson, A. P., K. D. Lafferty, A. M. Kuris, R. F. Hechinger, and W. Jetz. 2008. Homage to Linnaeus: How many parasites? How many hosts? *Proc. Natl. Acad. Sci.* 105:114821–11489.
 10. Lessler, J., Cummings, D. A. T. 2016. Mechanistic Models of Infectious Disease and Their Impact on Public Health, *American Journal of Epidemiology* 183(5):415–422

Chapter I: Vaccine-driven virulence evolution: consequences of unbalanced reductions in mortality and transmission and implications for pertussis vaccines

Foreword

This chapter was published as a research article in Journal of the Royal Society Interface in December of 2019:

Miller, I.F. and Metcalf, C.J.E. 2019. Vaccine-driven virulence evolution: consequences of unbalanced reductions in mortality and transmission and implications for pertussis vaccines. *J. R. Soc. Interface*.162019064220190642 <http://doi.org/10.1098/rsif.2019.0642>

Abstract

Many vaccines have heterogeneous effects across individuals. Additionally, some vaccines do not prevent infection, but reduce disease-associated mortality and transmission. Both of these factors will alter selection pressures on pathogens and thus shape the evolution of pathogen virulence. We use a mathematical modelling framework to show that (i) the balance of how vaccines reduce transmission versus mortality and (ii) individual variability in protection conferred both shape the evolution of pathogen virulence. Epidemiological (burden of disease) and evolutionary (pathogen virulence) outcomes are both worse when vaccines confer smaller reductions in transmission than in mortality. Furthermore, outcomes are modulated by variability in vaccine effects, with increased variability limiting the extent of virulence evolution but in some cases preventing eradication. These findings are pertinent to current concerns about the global resurgence of pertussis and the efficacy of pertussis vaccines, as the two classes of these vaccines may reduce disease symptoms without preventing infection and differ in their ability to reduce transmission. Furthermore, these findings point to the importance of generating precise predictions for virulence evolution in *Bordetella pertussis* (and other similar pathogens) by incorporating empirical characterizations of vaccine effects into models capturing the epidemiological details of this system.

Introduction

Vaccination is one of the most powerful public health tools available [1]. When vaccines block within and/or between host propagation of pathogens, they provide both individual benefits through their direct protective effects and population benefits ranging from herd immunity to eradication. However, vaccination can fundamentally change the ecology of infectious disease systems and introduce novel selective pressures that drive pathogen evolution, potentially reducing or negating these benefits. Pathogen life history traits can evolve in response to vaccination when pathogens retain the ability to infect some or all of the host population. One such pathogen life history trait is virulence, or the rate of disease associated host death. Virulence evolution has been a general focus in the fields of epidemiology and evolutionary biology [2–4] and has received particular attention in the context of vaccination.

Virulence determines a pathogen's effects on morbidity and mortality, as it describes the aggressiveness of the pathogen's host manipulation strategy [5]. When host death truncates transmission, virulence is inversely proportional to transmission time. Thus, virulence in itself is never adaptive, but it is often maintained as an unavoidable pleiotropic byproduct of transmission. Theoretical and empirical evidence points towards a saturating relationship between virulence and transmission, creating a trade-off that shapes pathogens' life history strategies [5–8]. This transmission-virulence trade-off has been shown to emerge from within-host processes [9], and is often reflected phenomenologically in population level evolutionary epidemiological models [10]. In most scenarios, intermediate virulence strategies confer the highest fitness because increased transmission rate comes at the price of increased host mortality and decreased transmission time [6, eqn. 1 below]. Here, we define virulence as the rate of disease associated mortality in hosts with no mortality blocking immunity, noting that the

operational definition of virulence varies between studies, and the term “virulence” itself is sometimes used to describe infectivity rather than aggressiveness in plant systems [5].

Vaccination can drive virulence evolution by inducing forms of immunity that alter the relationship between transmission rate and time. Some vaccines are thought to have an “anti-growth” effect (“ r_2 ” parameter in the models developed by Gandon et al. [11,12]) by slowing the within-host replication rate of a pathogen, leading to coupled reductions in both transmission and mortality. In a population level model, Gandon et al. [11] (see also [12]) found that vaccines that moderately reduce pathogen replication rate select for increased pathogen virulence, but lead to reductions in prevalence. Analogous within host models that consider transmission and mortality to be functions of within host pathogen density also predict that pathogens should evolve increased virulence when vaccine induced immunity impedes the replication of a pathogen [13,14], and that virulence should increase with increasing immune efficacy [15].

Other vaccines are thought to only block mortality effects without conferring any reduction in transmission. Gandon et al. [11] showed theoretically that the use of such mortality blocking (“ r_4 ” in their model specification) vaccines that do not reduce transmission can drive the evolution of very high pathogen virulence. Normally, highly virulent strategies confer low fitness because rapid host mortality truncates the transmission period. But when vaccines remove or reduce this cost of mortality, pathogens are able to increase their total fitness by increasing their transmission rate. Non-immunized individuals are disproportionately affected by the evolution of increased virulence—they experience drastically increased mortality while vaccinated individuals are at least partially protected. Several empirical examples support the predictions of Gandon et al. [11]. Read et al. [16] showed convincingly that when vaccines blocked the mortality effects of Marek’s disease virus in commercial poultry, the virus evolved

higher virulence to the point where vaccinated hosts lost all benefits of vaccine induced immunity and non-vaccinated individuals experienced increased mortality. Recently, Fleming-Davies et al. [17] showed that incomplete acquired immunity (analogous to vaccination) to the bacterial pathogen *Mycoplasma gallisepticum* in finches selects for increased virulence, as low-virulence strains are unable to infect previously infected hosts, while higher-virulence strains retain this ability.

Despite the development of a rich theoretical framework for investigating vaccine driven virulence evolution, this approach has not yet been applied to many important vaccine types. In particular, there is a clear knowledge gap about vaccines with unbalanced effects on transmission and mortality, whose effects are not fully or accurately captured by either the anti-growth or mortality blocking models of vaccine action. These unbalanced effects may be characteristic of vaccines targeted toward pathogen proteins like toxins that cause damage to the host, since toxins generally have a dual effect, reducing host survival (as considered in Gandon et al. [11]) but also increasing pathogen growth, often via immunosuppressive effects. As a result, toxin-targeting vaccines can slightly reduce transmission while also having significant mortality reducing effects. Investigating how such vaccines might drive virulence evolution requires a model that can consider both effects independently in the same framework. Previous model formulations are unsuitable for investigating this range of vaccine effects, because they either consider reductions in transmission and mortality to be necessarily coupled via a reduction in pathogen replication rate [e.g. 18], or assume that transmission is necessarily reduced to a greater degree than mortality by layering anti-growth and transmission blocking effects. Here, we present a modification to previous modeling frameworks that allows us to investigate and

compare between the effects of various modes of vaccine action on the evolution of pathogen virulence (fig. 1).

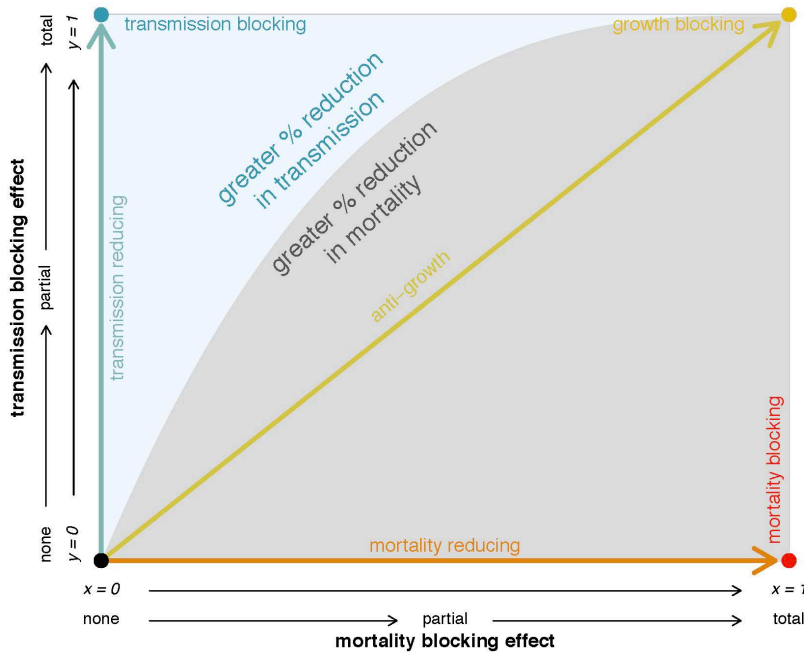


Figure 1: A model schematic encompassing multiple modes of vaccine action

In the model framework that we present, vaccine induced immunity can act in two non-exclusive ways. First, immunity can have a mortality reducing effect (x axis). Second, immunity can have a transmission reducing effect (y axis) by decreasing the within-host replication rate of the pathogen, which has a non-linear effect on transmission. Thus, our model allows us to consider not only “anti-growth” and “anti-toxin” effects, but also scenarios in which vaccines have unequal effects on transmission and mortality. An important aspect of this model is that immunity does not reduce the rate at which susceptible individuals become infected when challenged—consistent with the action of *B. pertussis* vaccines. The shaded regions show the parameter spaces that correspond to a greater percentage decrease in disease associated mortality rate or onward transmission rate for an individual treated with a vaccine. The boundaries of the shaded areas were generated from the equations for transmission and disease associated mortality given below, with $c_1=1.0$, $c_2=0.33$, and $i = 1.0$.

This model framework might provide particularly important insights into the *Bordetella pertussis* system. Both whole-cell pertussis vaccines (“wP”), which contain killed *B. pertussis* bacteria, and acellular pertussis vaccines (“aP”), which contain a small subset of the repertoire of proteins *B. pertussis* would normally present to the human immune system [19], are known to significantly reduce disease associated damage. However, evidence from a primate model system suggests that unlike naturally acquired immunity, neither form of vaccine induced immunity is able to prevent infection [20–22]. Epidemiological patterns suggest that both do moderately reduce onward transmission [23,24] but that aP vaccines reduce transmission to a lesser extent [25,26]. Thus, vaccinated individuals challenged by *B. pertussis* become asymptotically infected and can carry out some amount of transmission. The model framework presented in figure 1 is able to represent these effects, which are intermediate between anti-growth and anti-toxin.

Another aspect of vaccine driven virulence evolution that we seek to investigate relates to the structure of vaccine induced immunity in host populations. While the importance of characterizing “landscapes of immunity” and predicting their evolutionary and epidemiological effects is increasingly recognized [27], complexity has been conspicuously lacking in current modeling efforts, despite empirical evidence for its existence. To date, only the simplest distributions of immunity have been considered; almost all models (with the exception of [28–31]) consider only “binary” distributions of immunity, under the assumption that vaccination has a “fixed effect” in all individuals. Yet, it has long been known that vaccines can have a “variable effect,” creating continuous distributions of immunity within populations [32,33], and recent efforts have sought to characterize these patterns [34]. Variability in age, sex, or environmental factors, could create population level immunological heterogeneity in the absence of vaccination.

These factors could also contribute to heterogeneity in combination with vaccination or through interactions with vaccine efficacy.

Incorporating immunological heterogeneity into models could potentially reveal safe implementation strategies for mortality blocking vaccines, which have previously been found to have dangerous long-term effects as they drive the evolution of hypervirulent pathogens at any level of coverage. Gandon et al. [11] found that mortality blocking vaccines drove the evolution of hypervirulent pathogens while assuming that vaccination had fixed effect, creating a binary distribution of immunity in the host population (see fig. 2). The experimental work of Read et al. [16] that produced similar results used hosts populations that exhibited such homogeneity; all vaccinated individuals were age matched and kept in identical conditions to reduce the variance in vaccine effect. In both cases, the optimum pathogen virulence strategies in the two host categories were extremely different, especially when vaccination completely eliminated mortality effects. Highly virulent strategies emerged as pathogens gained most of their fitness through the vaccinated host group. Because there were no hosts with intermediate immunity, the costs of a hypervirulent strategy were only incurred in unvaccinated individuals. The few inquiries into the consequences of immunological heterogeneity for virulence evolution have indeed produced intriguing results. For example, Ganusov [28] found that in a within host model, heterogeneity in the lethal pathogen density slightly decreased the evolutionary stable degree of virulence.

Here, we apply adaptive dynamics to a non-system specific epidemiological model to quantify the extent to which patterns of vaccine driven virulence evolution and their associated epidemiological outcomes are dependent upon i) the relative strength of mortality and transmission reducing vaccine effects, and ii) variation in the effects of vaccination between

individuals. We find that drastically different evolutionary and epidemiological outcomes can result from the use of vaccines that differ only slightly in their effects on mortality and transmission and/or in their variability in effect among individuals, and that immunological variability can buffer against virulence evolution.

Methods

To investigate the evolutionary and epidemiological consequences of the use of vaccines that block mortality, transmission or a combination of the two, we constructed an evolutionary epidemiological model that follows a susceptible–infected–recovered (SIR) framework in which susceptible hosts become infected in a density-dependent fashion and then permanently recover with no chance of reinfection. We include two classes of infected hosts in order to explore competition between two pathogen strains of differing virulence and assume no coinfection or superinfection. The susceptible and infected host populations are structured by immunity. The relative frequencies of individuals born into each immunity class in the susceptible population are fixed, consistent with a constant vaccination rate and outcome and no host evolution. We vary how immunity affects transmission and mortality, and characterize the dynamics of virulence evolution as pathogens adapt to immunity distributions of various shapes using an adaptive dynamics approach. We then explore the epidemiological outcomes associated with evolutionarily stable pathogen virulence, as well as the potential for pathogen eradication. We note that these methods are not intended to make specific predictions about virulence evolution in *B. pertussis* or any other disease system. Rather, they are designed to explore how certain mechanistic details of vaccine action (that do differ between the two classes of pertussis

vaccines) might generally shape pathogen evolution. Definitions of the parameters and variables used throughout the paper are given in table 1.

Virulence and immunity

We assume a saturating relationship between virulence (α) and transmission (β):

$$(1) \quad \beta(\alpha, i) = c_1 ((1 - i y) \alpha)^{c_2}$$

The parameter y determines the relative effect of immunity on transmission. We assume that immunity (i) reduces transmission by scaling virulence rather than by scaling total transmission, consistent with immunity limiting the within host proliferation of pathogens. For all results presented in the main text, we set $c_1=1$ and $c_2=0.333$. We repeated our analyses with an alternate shape of the virulence-transmission trade-off curve ($c_1=1.2$, $c_2=0.45$) and found that our results were qualitatively similar (see figs. S1-2). We model disease associated mortality (v) as function of virulence and immunity, which takes values in the interval $[0,1]$ and scales the disease associated host mortality rate:

$$(2) \quad v(\alpha, i) = \alpha (1 - i x)$$

The parameter x determines the relative effect of immunity on disease associated mortality.

Both x and y are constrained to take values in the interval $[0,1]$. When $x > 0$ and $y = 0$, immunity reduces disease associated mortality but has no effect on transmission. When $x > 0$ and $y > 0$, immunity reduces both transmission and disease associated mortality. When $x = 1$ immunity completely blocks disease associated mortality, and when $y = 1$ immunity completely blocks transmission. When $x = 0$ and $y > 0$, immunity reduces transmission but has no effect on disease associated mortality. Here, we consider values of x and y in $\{0, 0.05, 0.5, 0.95, 1.0\}$.

Note that the relationship between the parameters x and y , and the relative percentage decrease in

transmission/mortality experienced *in a population*, does not necessarily map to the shaded regions in fig. 1 which represent *individual level* effects, as variable vaccine effects and vaccination rates less than 100% create immunological heterogeneity.

Table 1: Parameter and Variable Definitions

Parameter	Definition	Parameter	Definition
x	Mortality reducing effect of immunity	γ	Recovery rate
y	Transmission reducing effect of immunity	K	Density dependent growth scaling factor
i	Immunity	S_i	Number of susceptible individuals of immune class i
α	Virulence	$I_{i,1}$	Number of individuals of immune class i infected by resident pathogen
β	Transmission rate	$I_{i,2}$	Number of individuals of immune class i infected by invader pathogen
c_1, c_2	Shape parameters for virulence-transmission trade-off	R	Number of recovered individuals
v	Disease associated mortality rate	α_1, α_2	Virulence of the resident and invader pathogens
D_i	Density of immune class i in immunity distribution	$S_{0i}, I_{0i,1}, I_{0i,2}, R_0$	Initial number of individuals in each state class
θ	Vaccination rate	$\alpha_{ES,NV}$	Evolutionary stable virulence in the absence of vaccination
λ	Shape parameter for immunity distribution	α_{ES}	Evolutionary stable virulence in presence of vaccination
M	Set of immune categories	α_R	Virulence associated with repeller point
b	Birth rate	χ_i	Case fatality ratio of individuals of immune class i
μ	Mortality rate	$\chi_{all}, \chi_{vacc}, \chi_{non-vacc}$	Case fatality ratio of all individuals, vaccinated individuals, and non-vaccinated individuals

Host population immunity structures

We explore several forms of vaccine induced immunity distributions (fig. 2). We defined the immunity distributions created by vaccines with variable effects according to the following function, which gives the density of individuals with immunity i in the host population:

$$(1) \quad D_i(i, \theta, \lambda) = \begin{cases} \frac{(1-\theta) + \theta \lambda e^{-\lambda(1-i)}}{\sum_{i \in M} D_i} & \text{for } i = 0 \\ \frac{\theta \lambda e^{-\lambda(1-i)}}{\sum_{i \in M} D_i} & \text{for } i > 0 \end{cases}$$

M is the set of possible immunity categories, θ is the proportion of the population that is vaccinated, and λ is the shape parameter of the immunity distribution. For comparison, we also explored “binary” immunity distributions generated by vaccines with a fixed effect. We define such immunity distributions according to the following function, which gives the density of individuals with immunity i in the host population:

$$(2) \quad D_i(i, \theta) = \begin{cases} \theta - 1 & \text{for } i = 0 \\ \theta & \text{for } i = 1 \\ 0 & \text{for } i \notin \{0, 1\} \end{cases}$$

For all forms of vaccine induced immunity distributions considered, we considered vaccine coverages θ of $\{0, 0.05, 0.10, \dots, 1\}$. In this model formulation, incomplete vaccine efficacy (i.e. “leakiness”) can be considered by setting x or y to a value in the interval $(0, 1)$ rather than by constraining the maximum value of i to be less than 1.

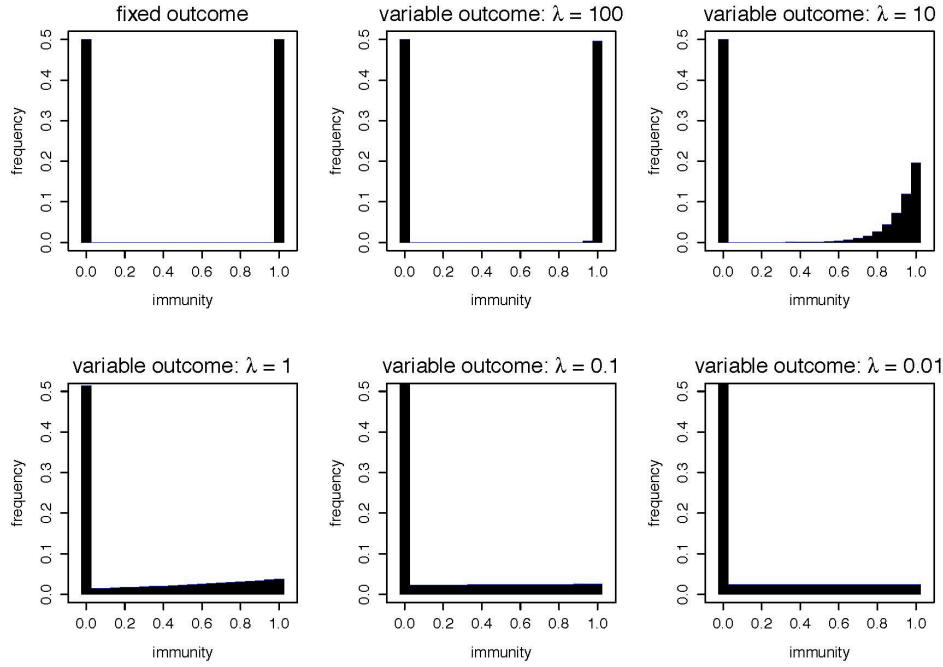


Figure 2: Immunity distributions

Vaccines that have a variable effect create continuous distributions of immunity (modeled here as pseudo-continuous distributions), while vaccines that have a fixed effect create a binary distribution of immunity. In all plots, $\theta = 0.5$

Epidemiological model

We conducted an evolutionary epidemiological investigation of the effects of vaccination on the evolution of virulence using compartmental epidemiological models [35]. The host population is partitioned into 21 immunity categories in the set $M = \{0, 0.05, 0.10, \dots, 0.95, 1.00\}$.

The dynamics of the host population are described by the following set of differential equations, in which the subscript i ($i \in M$) indicates immunity class. N is the total population size, S_i is the number of susceptible individuals, R is the number of recovered individuals. $I_{i,1}$ is the number of individuals infected with the resident pathogen strain (with virulence α_1), and $I_{i,2}$

is the number of individuals infected with the invader pathogen (with virulence α_2). In all analyses, we set the background mortality rate, μ , to 0.05, the birthrate, b , to 0.07, and the recovery rate, γ , to 0.1. These parameters are not intended to represent any particular disease system. We set K equal to $\frac{b}{b-\mu}$ so that the population size would be 1 at disease free equilibrium.

$$(1) \quad \frac{dS_i}{dt} = b N \left(1 - \frac{N}{K}\right) \frac{S_{0i} + I_{0i,1} + I_{0i,2} + R_{0i}}{N_0} - \mu S_i -$$

$$\sum_{j \in M} \beta(\alpha_1, j) S_i I_{j,1} - \sum_{j \in M} \beta(\alpha_2, j) S_i I_{j,2}$$

$$(2) \quad \frac{dI_{i,1}}{dt} = \sum_{j \in M} \beta(\alpha_1, j) S_i I_{j,1} - \mu I_{i,1} - v(\alpha_1, i) I_{i,1} - \gamma I_{i,1}$$

$$(3) \quad \frac{dI_{i,2}}{dt} = \sum_{j \in M} \beta(\alpha_2, j) S_i I_{j,2} - \mu I_{i,2} - v(\alpha_2, i) I_{i,2} - \gamma I_{i,2}$$

$$(4) \quad \frac{dR}{dt} = \gamma I_{i,1} + \gamma I_{i,2} - \mu R$$

We assume that the total number of births is proportional to the total size of the population, and that the proportion of births into each category is equal to the initial density of individuals in that category. This assumption is consistent with a constant vaccination rate and efficacy, where the vaccination status and efficacy of vaccination in a newly born individual are independent of the same traits of the parent. We assume density dependent transmission (number of new infections proportional to density of susceptible and infected individuals). We model transmission reducing immunity (γ) as reducing the rate at which infected hosts transmit to susceptible hosts, rather than as reducing the rate at which susceptible hosts become infected. This is consistent with our assumption that vaccination does not reduce the rate at which susceptibles become infected when challenged. For all analyses described below, we constructed the starting host population according to the following equations:

$$(5) \quad S_{0i} = 0.99 D_i(i, \theta)$$

$$(6) \quad I_{0_{i,1}} = 0.01 D_i(i, \theta)$$

$$(7) \quad I_{0_{i,2}} = 0$$

$$(8) \quad R_0 = 0$$

Evolutionary dynamics

We take an adaptive dynamics approach (see Brännström et al. [36] for an excellent introduction to this topic) to characterize how various distributions of mortality blocking immunity in the host population affect evolutionary dynamics and create evolutionary stable virulence strategies (ESS, Maynard Smith and Price [37]). While studies of virulence evolution often use R_0 as a proxy for pathogen fitness, the ESS virulence strategy is determined by a pathogens' invasion fitness (R_E , equivalent to effective reproductive number), which is dependent upon the composition of the host population in which the pathogen is evolving. Whenever hosts vary in their ability to resist infection, the relative frequency of susceptible hosts in each immunity category will change after the introduction of a pathogen. As a result, the R_E and R_0 values associated with a pathogen strategy are not equivalent, and an adaptive dynamics approach is required [38]. Since hosts do not vary in their ability to resist infection in our model, inferring the evolutionary stability of pathogen virulence strategies from their associated R_0 values is equivalent to an adaptive dynamics approach. However, we proceed with a formal adaptive dynamics approach for ease of interpretation, and to lay the foundations for extending our methods to scenarios in which vaccines do affect the susceptibility of hosts to infection.

We begin by introducing a “resident” pathogen with virulence α_1 into a disease-free host population and simulating epidemiological dynamics (details described below) until epidemiological equilibrium is reached. Next, at this equilibrium we calculate the invasion

fitness, (e.g. effective reproductive number) R_E for various invading pathogen virulence strategies (α_2) using “next generation” methods [39]. We assume no superinfection and no co-infection. We conducted all pairwise comparisons of resident and invader virulence strategies in $\{\{0, 0.005, \dots, 0.100\}, \{0.11, 0.12, \dots, 1.0\}, \{1.1, 1.2, \dots, 10\}, \{11, 12, \dots, 100\}\}$ for various population immunity distributions to generate pairwise invasibility plots (PIPs).

We found several recurring motifs in the pairwise invasibility plots (PIPs) generated in our adaptive dynamics analyses (fig. 3). Many combinations of vaccine coverage and shape parameter resulted in a convergent stable strategy (CSS, Eshel [40]), indicating that virulence will evolve to the same stable value regardless of its initial value (fig. 3A). Other combinations resulted in more complex evolutionary dynamics involving both an ESS and a repeller point (fig. 3B). We denote the virulence associated with a stable strategy (either CSS or ESS) as α_{ES} , the virulence associated with a stable strategy in an unvaccinated population as $\alpha_{ES,NV}$, and the virulence strategy at which a repeller point occurs as α_R . If the initial virulence value is greater than α_R , unbounded selection for progressively more virulent strategies would occur. If the initial virulence value is less than α_R , virulence would evolve towards α_{ES} . If the assumption that evolution occurs in small steps is relaxed, then the direction of selection could change rapidly around the repeller point; a pathogen population with a virulence slightly less than α_R could be invaded by a rare mutant with a significantly more virulent strategy, reversing the direction of selection and resulting in the evolution of hyper-virulence. Alternatively, a pathogen population with a virulence strategy only slightly higher than α_R could be invaded by a rare mutant with a significantly less virulent strategy, reversing progressive evolution towards higher virulence. The evolutionary dynamics around a region of unviable virulence strategies adjacent to a repeller point (“repeller region” motif, fig.3C) would be expected to be similar to those around a repeller

point, except that large evolutionary “jumps” in virulence would be required to start or stop evolution towards hypervirulence. The fourth motif that we observed was selection for hypervirulence without any stable strategy (fig. 3D).

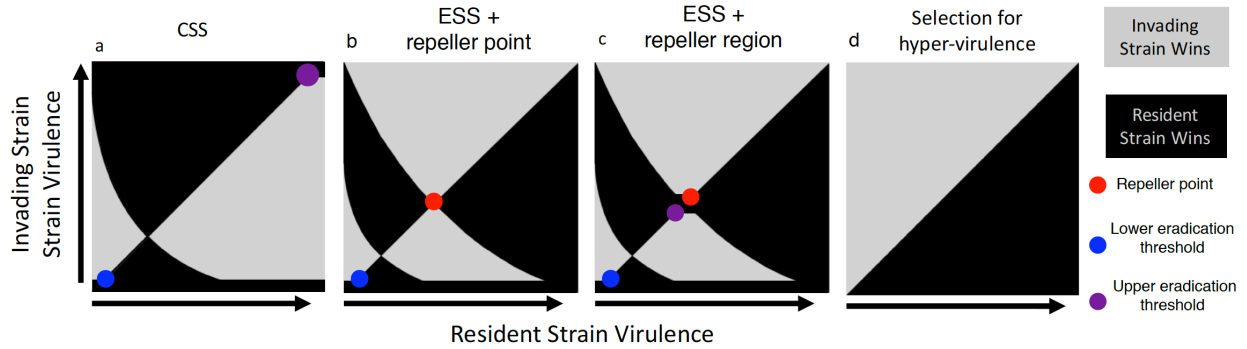


Figure 3: Pairwise invasibility plot motifs and eradication thresholds

Panels show four PIP motifs identified in the analyses of virulence evolution. A fifth motif (not shown) was found when no virulence strategy could invade, resulting in pathogen eradication.

PIPs also provide a means for assessing the evolutionary durability of a vaccine’s effect on pathogen eradication. When a vaccine is introduced into an infected population, “evolution-proof” eradication of the pathogen will occur if no virulence strategy will allow for its persistence. Eradication could also occur if the virulence strategy $\alpha_{ES,NV}$ is not viable after vaccine introduction, but some other virulence strategy is. We term this scenario “non-evolution-proof eradication.” This would occur if $\alpha_{ES,NV}$ falls below the lower eradication threshold in fig. 3A-C, above the upper eradication threshold in fig. 3A, or between the upper eradication threshold and repeller point in 3C. For a pathogen population to escape non-evolution-proof eradication, it must evolve a viable virulence strategy through a single large evolutionary step.

Epidemiological consequences

We investigated the long-term epidemiological consequences of implementing vaccines that create various distributions of immunity. For each immunity distribution considered, we found the ES pathogen virulence as described above. When an immunity distribution was predicted to drive the evolution of hyper-virulence, we used $\alpha=10^6$ to calculate epidemiological consequences. Such an extreme value of α may be biologically unattainable, but we found that epidemiological consequences were qualitatively and quantitatively similar when we repeated our analyses while setting $\alpha=10^2$ for all cases in which hypervirulence evolution occurred (results not shown). For each immunity distribution, we calculated the prevalence of the pathogen at epidemiological equilibrium (see computation methods below), and the case fatality rate (CFR), χ , experienced by vaccinated and unvaccinated individuals, as well as by the entire host population. We calculate the CFR for a population as the average CFR experienced by a new individual entering the population weighted by the proportion of new individuals entering each immunity class. This differs from the average CFR experienced by an individual in a population weighted by the proportion of individuals in each immunity class, as differences in disease associated mortality between immunity classes result in the distribution of immunity within the infected class differing from the distribution of immunity among individuals entering the population. CFRs were calculated for each immune class as:

$$(1) \quad \chi_i = \frac{v(\alpha_{ES,i})}{v(\alpha_{ES,i}) + \mu + \gamma}$$

The CFRs of all, vaccinated, and non-vaccinated individuals were calculated as average CFRs of the relevant immunity categories weighted by the proportion of relevant individuals in each category:

$$(2) \quad \chi_{all} = \sum_{i \in M} \frac{v(\alpha_{ES,i})}{v(\alpha_{ES,i}) + \mu + \gamma} D(i)$$

$$(3) \quad \chi_{vacc} = \frac{\frac{v(\alpha_{ES,0})}{v(\alpha_{ES,0}) + \mu + \gamma} (D_0 - (1 - \theta)) + \sum_{i \in (M > 0)} \frac{v(\alpha_{ES,i})}{v(\alpha_{ES,i}) + \mu + \gamma} D_i}{\theta}$$

$$(4) \quad \chi_{non-vacc} = \frac{v(\alpha_{ES,0})}{v(\alpha_{ES,0}) + \mu + \gamma}$$

Computational methods

We implemented the epidemiological model, and carried out all analyses in the R statistical software package [41]. We used the ‘deSolve’ package [42] to simulate the epidemiological dynamics of the system. For all epidemiological simulations we ran the model for 2000 time units (with a step size of 0.1), and checked that the system had reached epidemiological equilibrium (constant numbers of infected, susceptible and recovered individuals). The code associated with the analyses is available via Dryad.

Code accessibility

The code associated with the analyses presented in this chapter is available via Dryad (<https://doi.org/10.5061/dryad.6m905qfw1>).

Results

Evolutionary outcomes

We first investigated the evolution of virulence using pairwise invasibility analyses while varying vaccine coverage, the strength of mortality blocking effects (x), and strength of transmission blocking effects (y), for both variable and fixed effect vaccines. We also varied the shape of the immunity distribution (λ) for variable effect vaccines. For all combinations of mortality and transmission reducing effects, we found that evolutionary outcomes for variable effect vaccines approached those for fixed effect vaccines as the variation in vaccine effect dropped (i.e. as λ increased). When vaccine induced immunity did not completely block disease associated mortality ($x < 1$), we found that virulence always evolved towards a CSS, or that the pathogen was eradicated when immunity also greatly reduced transmission ($y \geq 0.95$, fig. 4). We found that evolution-proof eradication occurred when vaccine coverage was high, and when the variability of the vaccine effect was low. However, when vaccine coverage was high but eradication was not achieved, vaccines with less variable effects led to the evolution of more virulent pathogens (fig. 5). Similar results were obtained when vaccines completely blocked transmission and mortality effects ($x, y = 1$). This demonstrates how the outcome of pathogen life history evolution can be extremely sensitive to the shape of a vaccine induced immunity distribution.

When vaccines completely blocked mortality but not transmission, ($x = 1, y < 1$) the evolutionary outcome was highly dependent upon the shape of the immunity distribution and vaccine coverage. As coverage increased, and the effect of the vaccine became less variable, repeller points and regions emerged. The potential for evolutionary “jumps” in virulence to result

in the evolution of hypervirulence also increased as vaccine effects became less variable and coverage rose, as the repeller points moved closer to the ES virulence strategies (fig. 6). For very high vaccine coverage, and low vaccine effect variability, evolutionary stable virulence strategies disappeared, and evolution towards hypervirulence always occurred.

These results illustrate how heterogeneity in the strength of mortality blocking immunity can buffer against the evolution of increased virulence or hypervirulence. However, when immunity also acts to completely block transmission ($y=1$), this effect comes at the cost of pathogen eradication.

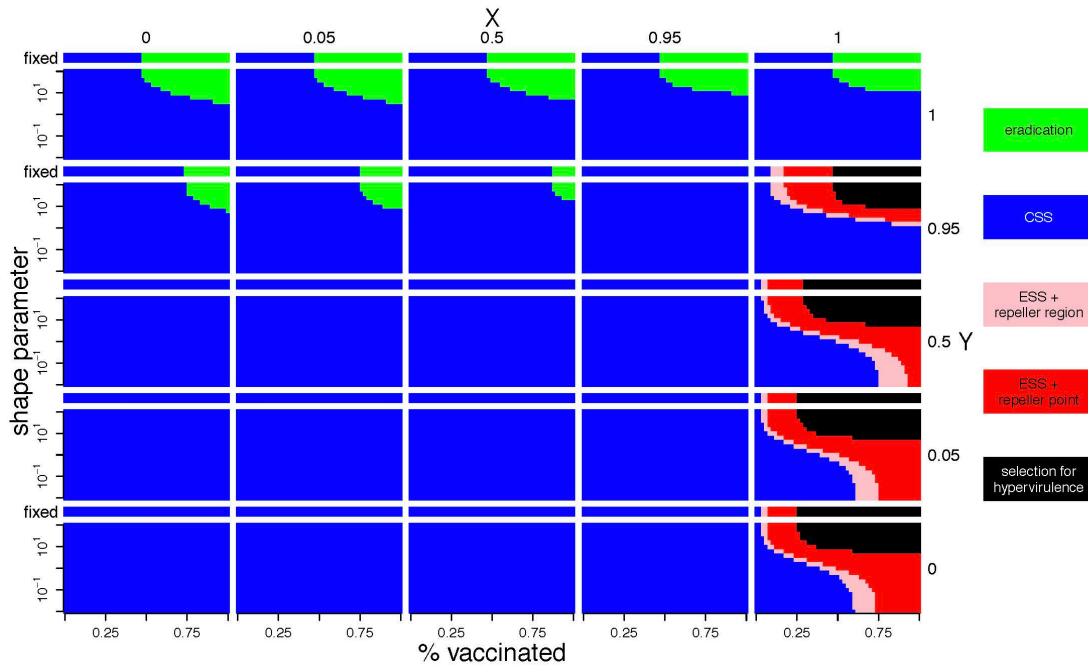


Figure 4: Adaptive dynamics results

The evolutionary motifs found for various combinations of vaccine coverage, variability in effects, and mortality and transmission reducing efficacy are shown. Green regions indicate scenarios in which evolution-proof eradication is achieved and virulence evolution cannot lead to pathogen persistence. Scenarios in which non-evolution-proof eradication is possible were found on the boundaries of the evolution-proof eradication regions (fig. S3). Lower and upper eradication thresholds are shown in figs. S3-4. Qualitatively similar results were found when assuming an alternate shape of the virulence-transmission trade-off curve (figure S1).

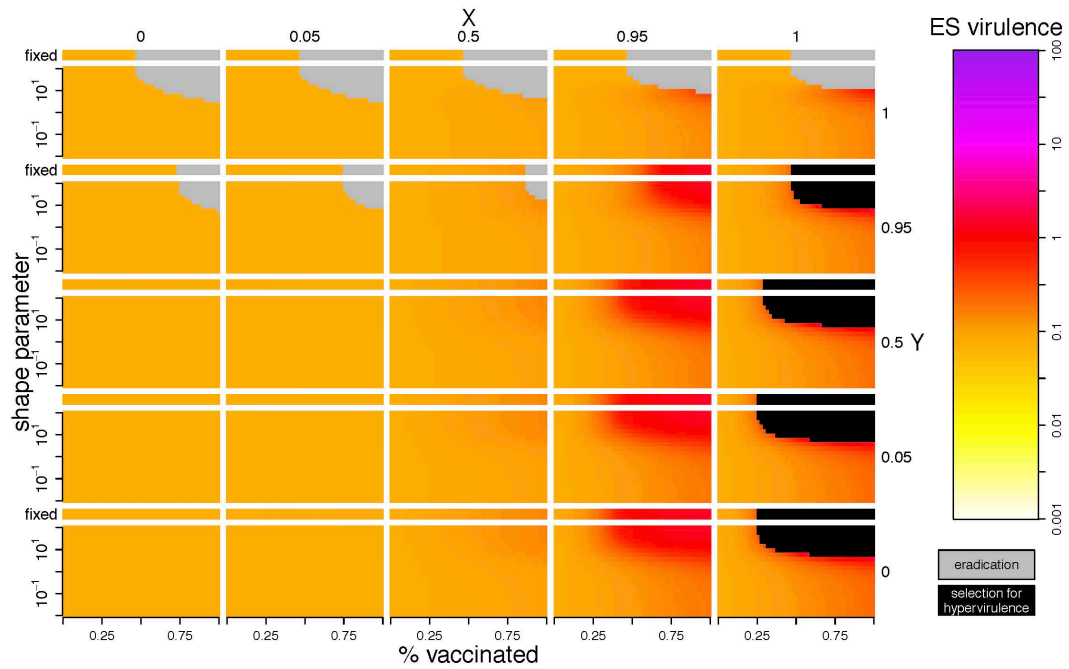


Figure 5: Evolutionary stable virulence (α_{ES})

Colors show the evolutionary stable virulence strategy (α_{ES}). No virulence strategy was evolutionary stable when either eradication or selection for hypervirulence occurred. Qualitatively similar results were found when assuming an alternate shape of the virulence-transmission trade-off curve (figure S2).

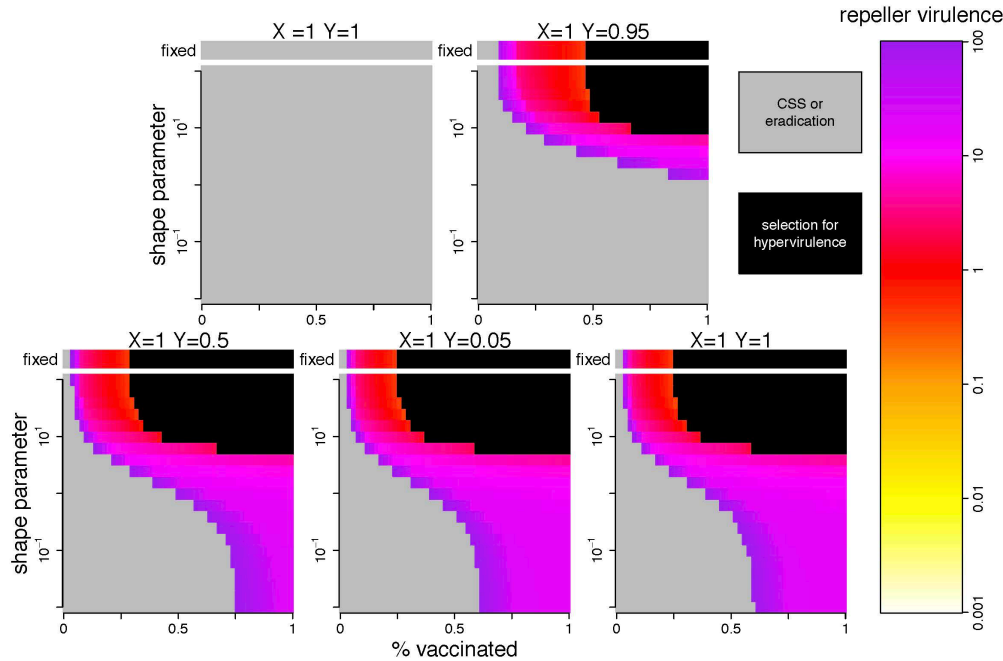


Figure 6: Repeller point virulence (α_R)

Colors show the level of virulence at which a repeller point or region was found (α_R). No repeller points or regions were found for other combinations of mortality (x) and transmission (y) blocking effects.

Epidemiological outcomes

Next, we investigated the epidemiological outcomes of virulence evolution driven by vaccination. Again, we found that the outcomes of variable effect vaccines approach those of fixed effect vaccines as variation in vaccine effect decreased (as one would hope). When vaccines had an anti-growth effect or reduced transmission to an equal or greater degree than mortality effects ($x \leq y$), we found that vaccines always reduced the average CFR experienced in a population (figure 7), decreased infection prevalence (figure 8), and decreased the rate of pathogen spread (i.e. decreased R_E , figure S8). The magnitude of these reductions was greater for vaccines that reduced transmission to a greater degree, and mortality to a lesser degree (figs. 7,8). Greater reductions in CFR were achieved at higher vaccine coverage, and lower variation in

vaccine effect (fig. 7). Unsurprisingly, reductions in CFR were mostly driven by a reduction in CFR amongst vaccinated individuals (figure S6, S7). In fact, CFR increased amongst unvaccinated individuals whenever the mortality blocking effect of the vaccine was strong ($0.5 < x < y$), except when the pathogen was eradicated. However, we did observe that when the mortality blocking effect was weak in both an absolute and relative sense or non-existent ($x < 0.5$ and $y > x$), reductions in average CFR were achieved without increases in CFR amongst unvaccinated individuals (fig. 7, S7).

When vaccines' mortality reducing effects were strong ($x \geq 0.5$) and greater than transmission reducing effects ($x > y$), we found that epidemiological outcomes were highly dependent upon vaccine coverage and the shape of the vaccine induced immunity distribution. In this parameter space, we found that vaccines always increased the potential for the pathogen to spread (fig. S8) but decreased infection prevalence in the population in which they were implemented unless selection for hypervirulence occurred (fig. 8). Surprisingly, we found that the greatest reductions in prevalence occurred just outside of the parameter space corresponding to the evolution of hypervirulence and increased prevalence. This further demonstrates how the population level consequences of vaccination are extremely sensitive to variation in vaccines' effect. When vaccines drove the evolution of hypervirulent pathogens, a drastic increase in CFR occurred unless vaccine coverage was very high, and the variation in vaccine effect was low or non-existent (fixed effect), in which case a reduction in average CFR (fig. 7) was obtained via a drastic reduction in CFR amongst vaccinated individuals (fig. S6) but at the cost of a drastic increase in CFR amongst unvaccinated individuals (fig. S7). A similar result was obtained at high vaccine coverage for $x=0.95$ and $y=0.5$, although the evolution of hypervirulence did not occur in this case.

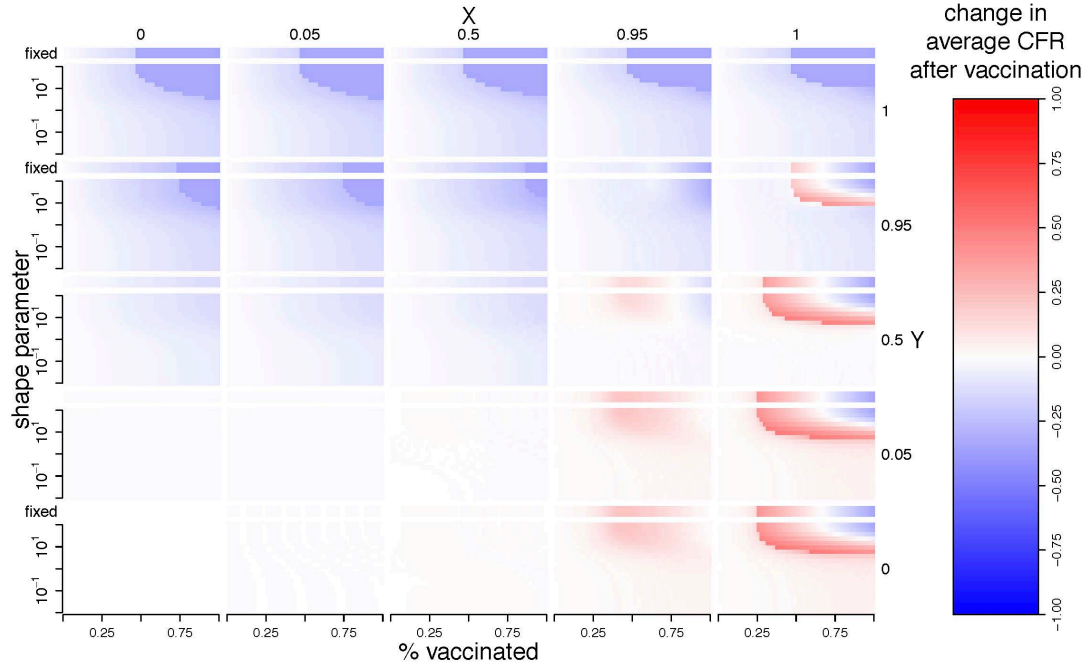


Figure 7: Change in case fatality rate after vaccine driven virulence evolution

The change in CFR was calculated as the difference between the average CFR experienced in a vaccinated population with $\alpha=\alpha_{ES}$ and the average CFR experienced in an unvaccinated population with $\alpha=\alpha_{ES,NV}$.

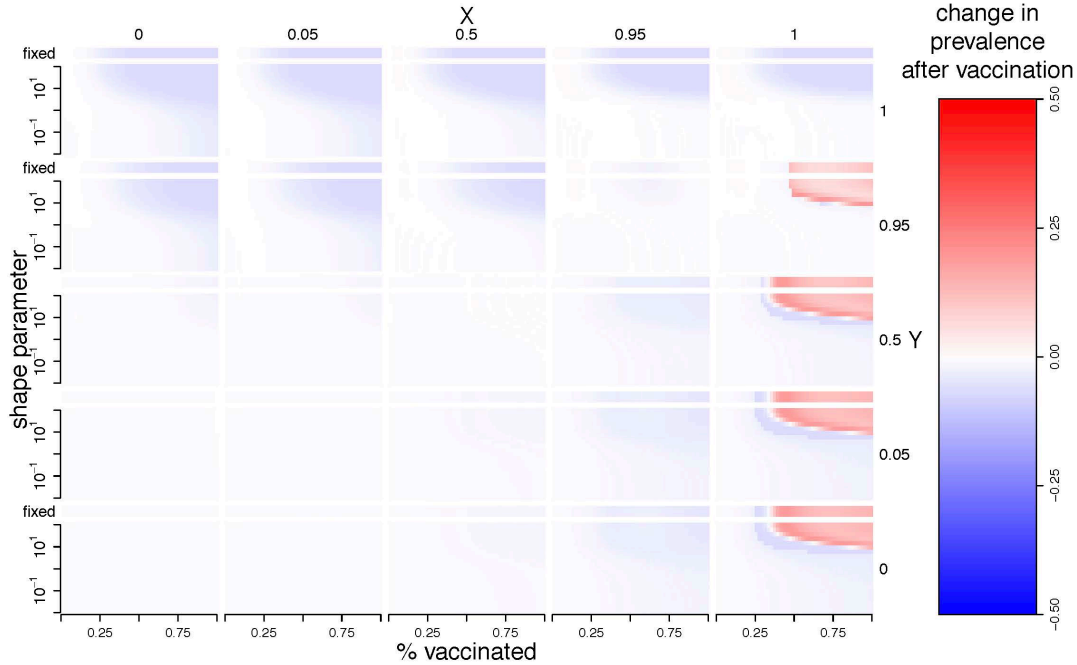


Figure 8: Change in equilibrium prevalence after vaccine driven virulence evolution

The change in prevalence at epidemiological equilibrium was calculated as the difference between the equilibrium prevalence experienced in a vaccinated population with $\alpha=\alpha_{ES}$ and equilibrium prevalence in an unvaccinated population with $\alpha=\alpha_{ES,NV}$.

Discussion

We investigated how the strength of mortality and transmission reducing vaccine effects and the degree of variability in individual outcomes of vaccination affect evolutionary and epidemiological patterns. Our results show that long term outcomes of vaccination are positive when transmission is reduced to a greater degree than mortality. In these scenarios vaccination always reduces the overall case fatality rate (CFR) and infection prevalence. We also found that when mortality reducing effects were high, but still less than transmission reducing effects, slight changes in the variability of individual outcomes of vaccination could determine whether the pathogen is eradicated, or evolves increased virulence (relative to the evolutionary stable

virulence in the absence of vaccination) resulting in increased CFR among unvaccinated individuals.

Vaccines which reduce mortality effects to a greater degree than transmission effects result in relatively worse evolutionary and epidemiological outcomes. For these vaccines, reductions in overall CFR are only achieved when variation in individual outcomes of vaccination is low and vaccines coverage is close to 100%. We found that these vaccines cannot concurrently reduce CFR and prevalence, and that any reduction in overall CFR comes at the cost of increased CFR amongst unvaccinated individuals. When vaccination completely blocked mortality but not transmission, evolutionary and epidemiological outcomes were highly sensitive to the variation in vaccine outcome, as slight changes in variability could switch evolutionary patterns from selection towards an evolutionary stable intermediate virulence strategy to selection for hypervirulence. Notably, we found that immunological variability buffers against the evolution of hypervirulence when immunity reduces mortality, but can also prevent pathogen eradication when immunity also completely blocks transmission. These results highlight the critical role that variation in vaccine outcome plays in driving pathogen evolution.

Beyond this more complete understanding of how relative vaccine effects on transmission vs. mortality shape virulence evolution, our findings show that accounting for variation in vaccine effects qualitatively changes the predictions of evolutionary epidemiological models. This suggests that considering immunological heterogeneity within populations will be important in any investigations into unintended evolutionary consequences of vaccination, especially in those considering other mechanisms of vaccine-induced immunity (e.g. transmission blocking, recovery expediting). Data on variation in vaccine effects is extremely limited or non-existent in most cases. Collecting such data, or leveraging existing data sets to

quantify this variation is an important research direction, with potential to improve predictions about both the short term epidemiological consequences and long term evolutionary outcomes associated with disease control efforts. For the latter, a broader scope of models will be required, potentially building on the development of new statistical methods for incorporating continuous heterogeneity into epidemiological models [31]. Together, these new data sets and modified methods should be used to both assess the evolutionary consequences of current vaccine use, and to evaluate the evolutionary impacts of vaccines in development.

We emphasize that regardless of their potential to drive the evolution of increased virulence, vaccines that reduce mortality effects to any degree always provide a personal benefit to recipients. The magnitude of this benefit is dependent upon the virulence of the pathogen and may vary between individuals if the vaccine has a variable effect. However, the rational choice for an individual in any scenario is to maximize protection against disease related mortality by being vaccinated.

Our model generates valuable insights for the pertussis system. Both whole-cell pertussis vaccines (wP) and acellular pertussis vaccines (aP) completely block mortality and moderately reduce transmission, but transmission is reduced to a greater degree by whole-cell vaccines [22]. We found that when vaccines completely block mortality effects, the evolutionary stable degree of virulence increases and the potential for runaway evolution towards hypervirulence rises as transmission reducing effects become weaker. While our model was not parameterized for pertussis and lacked many important epidemiological details of the system that could modulate patterns of virulence evolution, including waning immunity and differential mixing patterns, these results do suggest that the use of aP vaccines is riskier than the use of wP vaccines,

especially when the population immunity traits (vaccine coverage, shape of immunity distribution) are unknown or expected to change.

Definitive evidence for the recent evolution of *B. pertussis* [43,44] (including virulence evolution [45]), the global resurgence of pertussis incidence [46–48], and the high worldwide coverage of pertussis vaccines [49] all point to clear importance of modifying our general model to specifically address the epidemiology and biology of the *B. pertussis* system in order to validate and refine our predictions about vaccine driven virulence evolution. Major challenges in this endeavor include quantifying the relative transmission and mortality reducing effects of pertussis vaccines, and establishing which, if any, life history trade-off limits pertussis virulence evolution. *B. pertussis* related deaths are very uncommon in the United States and other developed countries [50], and mostly occur in unvaccinated infants who are unlikely to contribute significantly to transmission [19]. In these locations, it is plausible that a trade-off with the metabolic costs of toxin production [18] is the major factor limiting virulence evolution. In developing countries where *B. pertussis* mortality rates are much higher [50], a virulence-transmission trade-off might be more significantly involved. Future research about vaccine driven virulence evolution in *B. pertussis* should also investigate the time scale of virulence evolution so that vaccines can be compared in terms of both the magnitude and immediacy (or perhaps temporal irrelevancy) of their projected outcomes.

More broadly, our results have implications for natural populations in which transmission and/or mortality reducing immunity is not the product of vaccination. Variation among individuals in terms of immunity due to physiological or eco-immunological factors could potentially drive the evolution of pathogen virulence. Quantifying the extent and basis of immune function variation in natural populations will be an essential first step in establishing the

degree to which this occurs. If variation in immune function has a genetic basis, then host and pathogen populations will co-evolve. The model we present in this paper could be expanded to investigate how coevolutionary patterns affect the evolution of virulence.

In conclusion, we found that small differences in vaccines' effects on mortality and transmission can correspond to large differences in evolutionary and epidemiological outcomes. Likewise, we found these results to be highly sensitive to vaccine coverage and the shape of the immunity distributions that they create in populations. Immunological heterogeneity can buffer against virulence evolution when immunity reduces mortality, but can also hinder pathogen eradication when immunity completely blocks transmission. These findings point to the importance of measuring characteristics of vaccine's protective efficacy beyond their ability to provide personal protection, especially when they are thought to reduce mortality to a much greater degree than transmission, as is the case for pertussis vaccines.

Supplementary figures

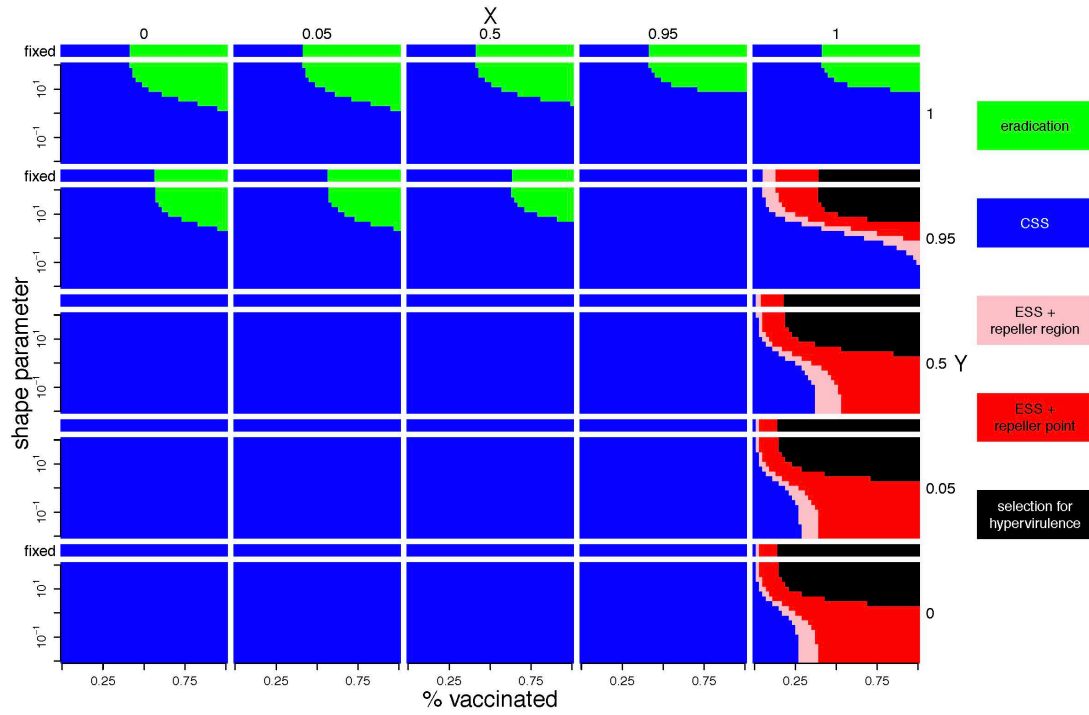


Figure S1: PIP results for alternate trade-off shape

Figure S1 shows PIP results for an alternate shape of the virulence-transmission trade-off curve ($c_1=1.2$, $c_2=0.45$). Labels are as in figure 4.

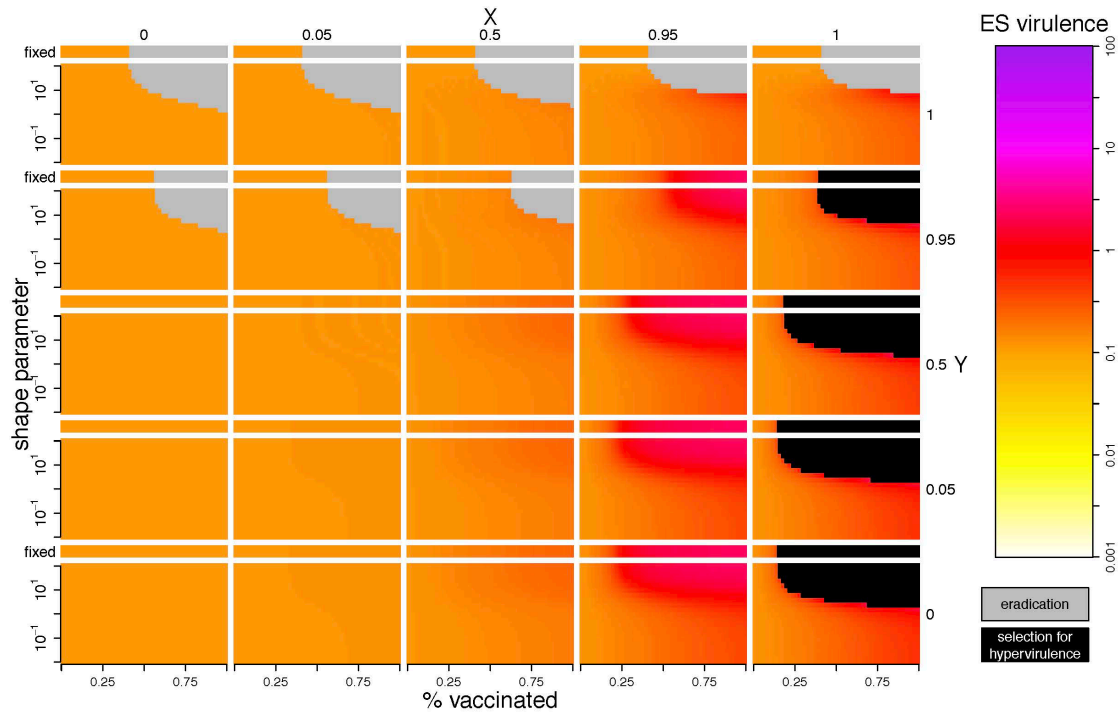


Figure S2: Evolutionarily stable virulence (α_{ES}) for alternate trade-off shape

Figure S1 shows α_{ES} for an alternate shape of the virulence-transmission trade-off curve ($c_1=1.2$, $c_2=0.45$). Labels are as in figure 5.

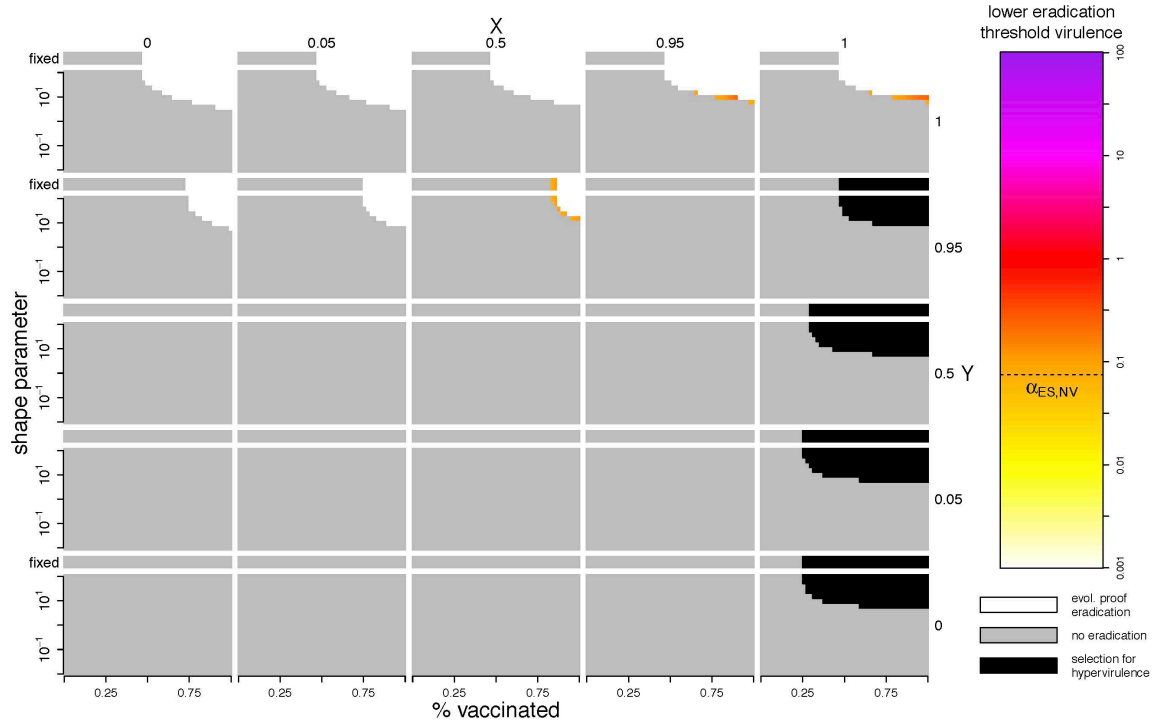


Figure S3: Non-evolution-proof eradication

Figure S3 shows the virulence level of the lower eradication bound for all parameter combinations that result $\alpha_{ES,NV}$ falling below the lower eradication bound, thus leading to non-evolution-proof eradication. No parameter combinations resulted in non-evolution proof eradication as a result of $\alpha_{ES,NV}$ falling above the upper eradication bound or between the upper eradication bound and repeller point.

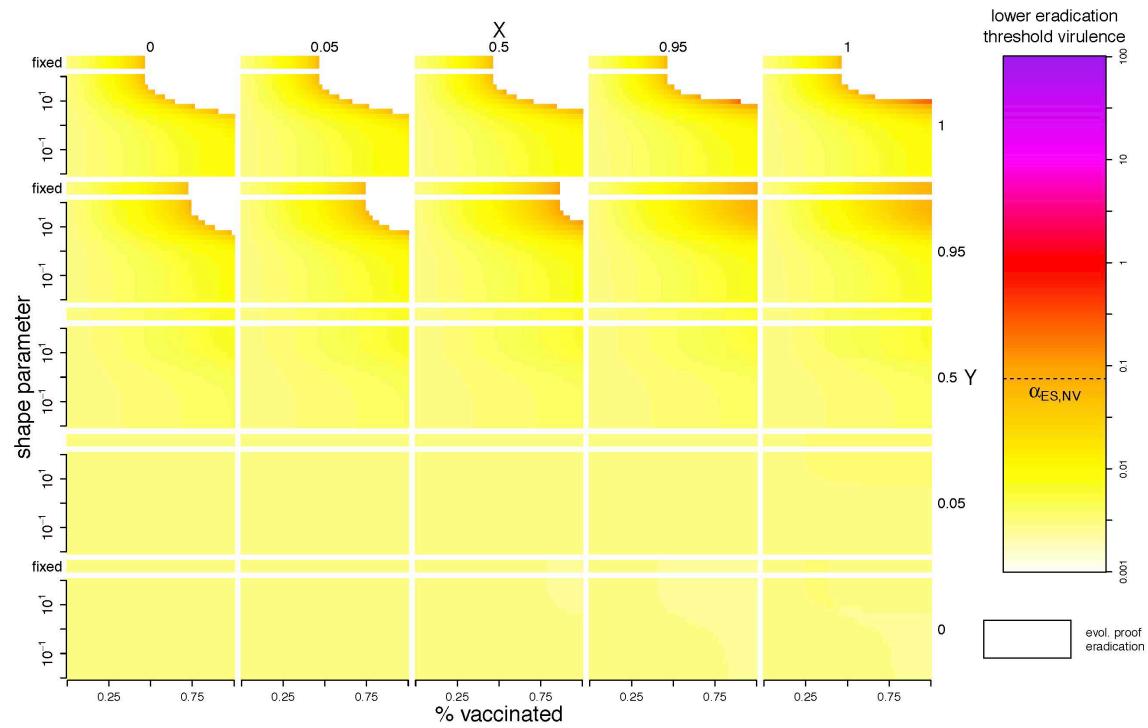


Figure S4: Lower eradication thresholds

Figure S4 shows the level of virulence at which lower eradication thresholds occur for various combinations of vaccine coverage, variability in effects, and mortality and transmission reducing efficacy.

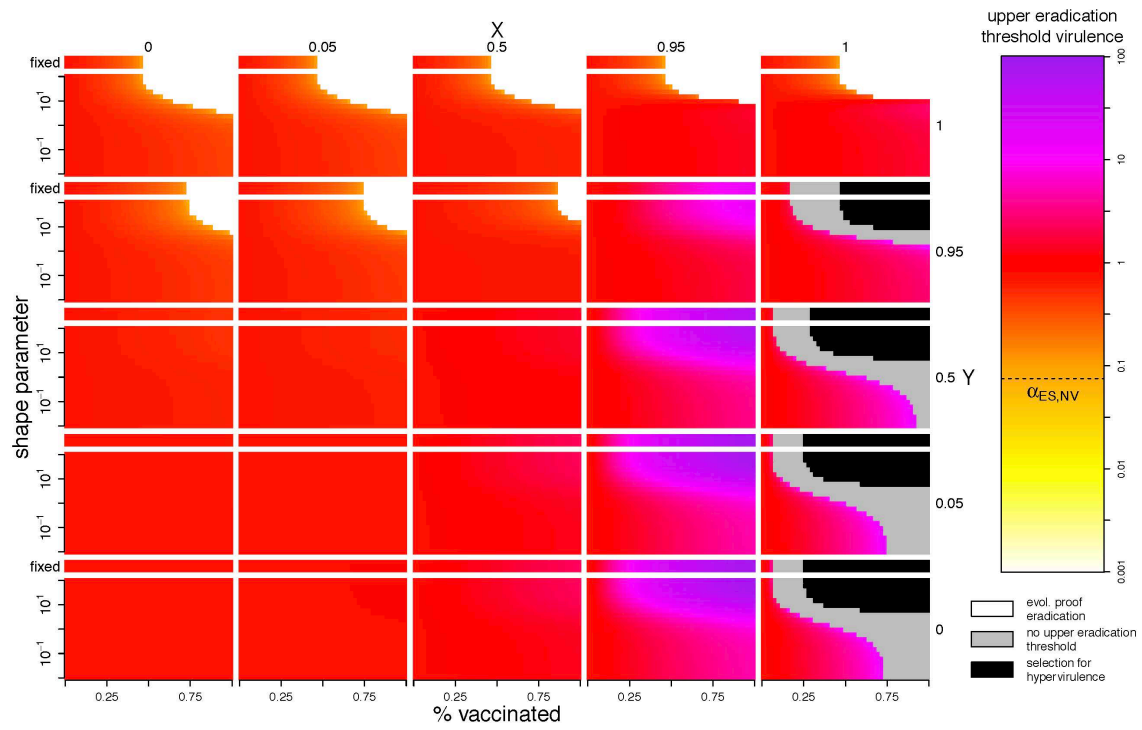


Figure S5: Upper eradication thresholds

Figure S5 shows the level of virulence at which upper eradication thresholds occur for various combinations of vaccine coverage, variability in effects, and mortality and transmission reducing efficacy.

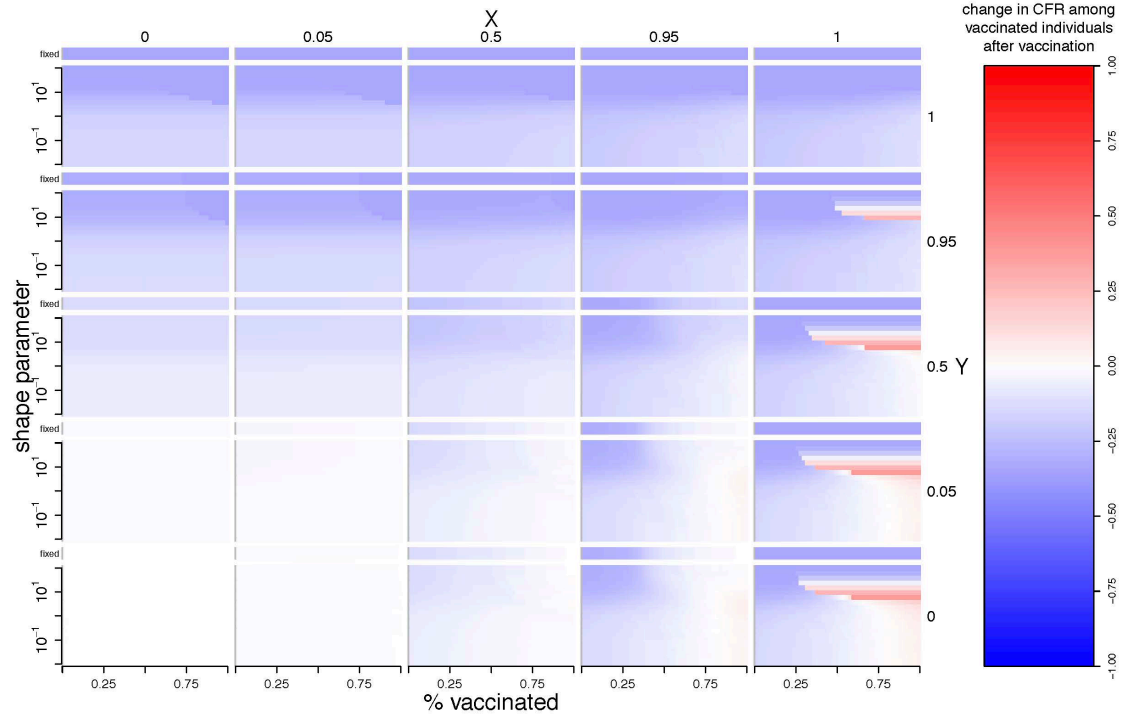


Figure S6: Change in CFR among vaccinated individuals after vaccine driven virulence evolution

The change in CFR was calculated as the difference between the average CFR experienced among vaccinated individuals in a vaccinated population with $\alpha=\alpha_{ES}$ and the average CFR experienced in an unvaccinated population with $\alpha=\alpha_{ES,NV}$.

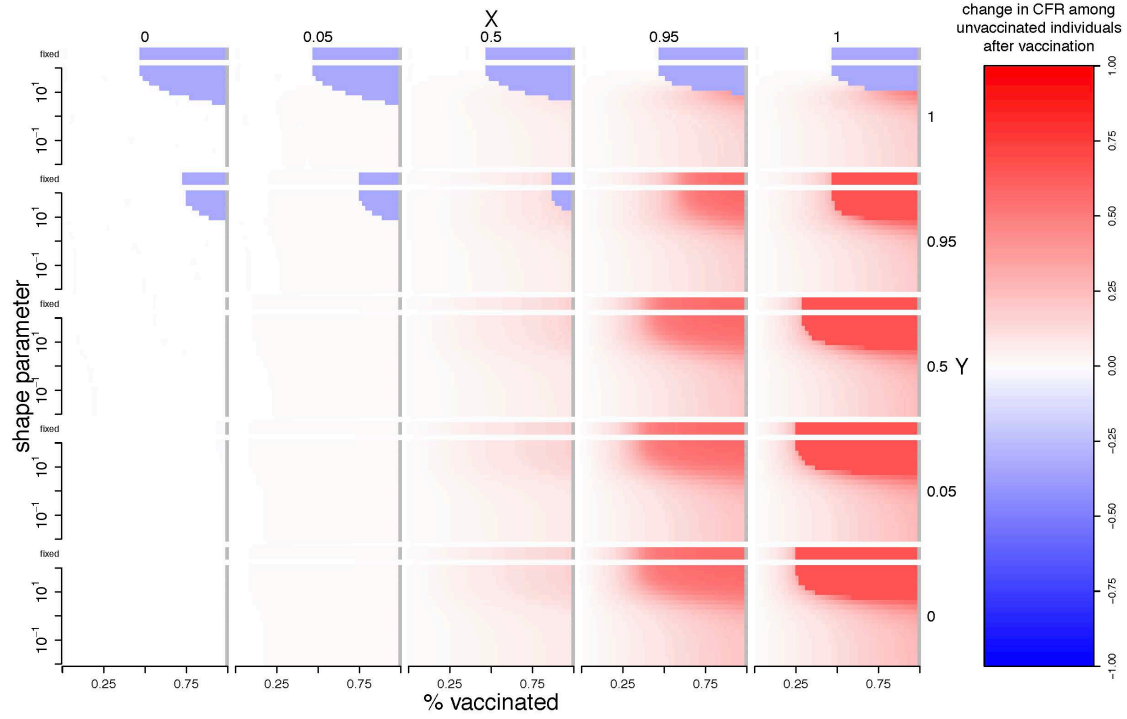


Figure S7: CFR among unvaccinated individuals after vaccine driven virulence evolution

The change in CFR was calculated as the difference between the average CFR experienced among unvaccinated individuals in a vaccinated population with $\alpha=\alpha_{ES}$ and the average CFR experienced in an unvaccinated population with $\alpha=\alpha_{ES,NV}$.

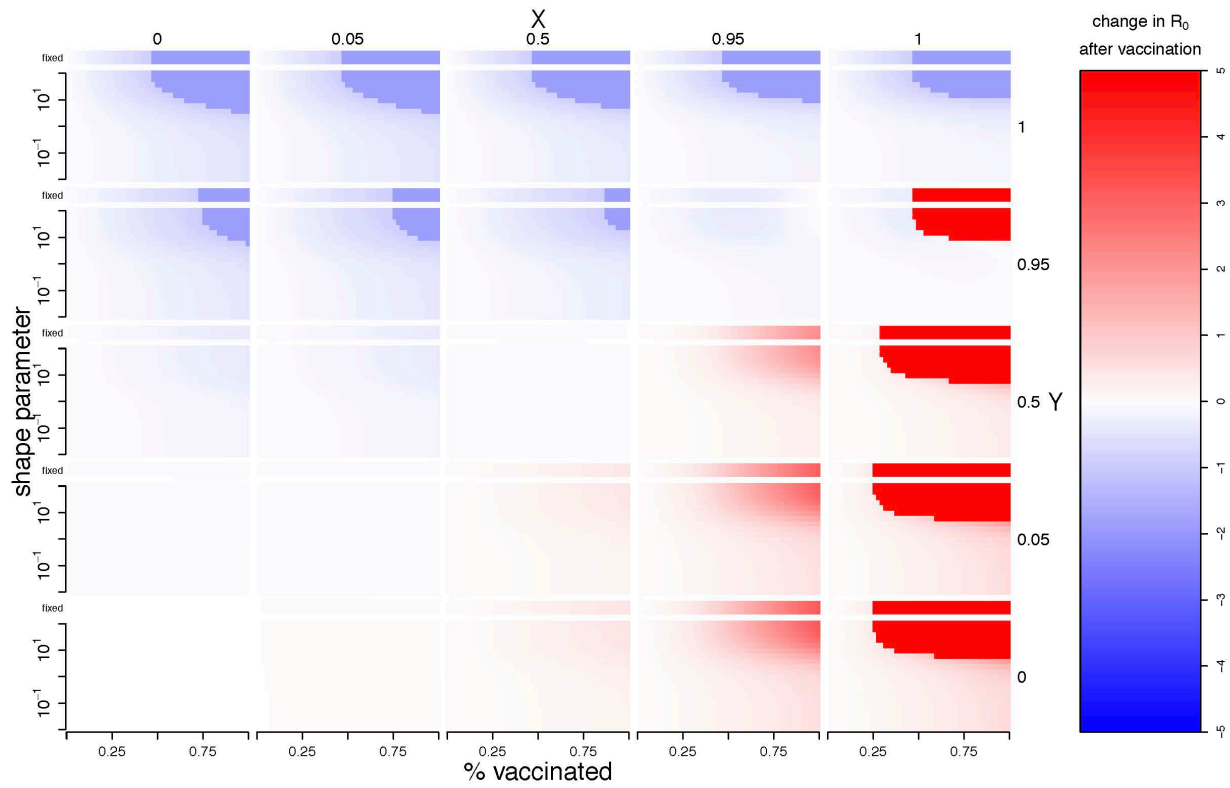


Figure S8: Change in R_0 after vaccination.

The change in R_0 after vaccination was calculated as the difference between the R_0 value associated with α_{ES} in a vaccinated population and the R_0 value associated with $\alpha_{ES,NV}$ in an unvaccinated population.

References

1. Roush SW, Murphy T V, The Vaccine-Preventable Disease Table Working Group. 2007 Historical Comparisons of Morbidity and Mortality for Vaccine-Preventable Diseases in the United States. *JAMA* **298**, 2155. (doi:10.1001/jama.298.18.2155)
2. Ebert D, Bull JJ. 2003 Challenging the trade-off model for the evolution of virulence. *Trends Microbiol.* **11**, 15–20. (doi:10.1016/S0966-842X(02)00003-3)
3. Porco TC, Lloyd-Smith JO, Gross KL, Galvani AP. 2005 The effect of treatment on pathogen virulence. *J. Theor. Biol.* **233**, 91–102. (doi:10.1016/j.jtbi.2004.09.009)
4. Diekmann U, Metz JAJ, Sabelis MW, Sigmund K, editors. 2002 *Adaptive Dynamics of Infectious Diseases: In Pursuit of Virulence Management*. Cambridge: Cambridge University Press.
5. Alizon S, Hurford A, Mideo N, Van Baalen M. 2009 Virulence evolution and the trade-off hypothesis: History, current state of affairs and the future. *J. Evol. Biol.* **22**, 245–259. (doi:10.1111/j.1420-9101.2008.01658.x)
6. Levin S, Pimentel D. 1981 Selection of Intermediate Rates of Increase in Parasite-Host Systems. *Am. Nat.* **117**, 308–315.
7. Anderson RM, May RM. 1982 Coevolution of Hosts and Parasites. *Parasitology* **85**, 411–426. (doi:10.1017/S0031182000055360)
8. Ewald PW. 1983 Host-parasite relations, vectors, and the evolution of disease severity. *Ann. Rev. Ecol. Syst* **14**, 465–85.
9. Alizon S, van Baalen M. 2005 Emergence of a convex trade-off between transmission and virulence. *Am.Nat.* **165**, E155–E167. (doi:doi:10.1086/430053)
10. Gandon S, Day T. 2007 The evolutionary epidemiology of vaccination. *J. R. Soc.*

- Interface* **4**, 803–817. (doi:10.1098/rsif.2006.0207)
11. Gandon S, Mackinnon MJ, Nee S, Read AF. 2001 Imperfect vaccines and the evolution of pathogen virulence. *Nature* **414**, 751–756. (doi:10.1038/414751a)
 12. Gandon S, Mackinnon M, Nee S, Read A. 2003 Imperfect vaccination: some epidemiological and evolutionary consequences. *Proc. R. Soc. B Biol. Sci.* **270**, 1129–1136. (doi:10.1098/rspb.2003.2370)
 13. André J-B, Gandon S. 2006 Vaccination, within-host dynamics, and virulence evolution. *Evolution (N. Y.)*. **60**, 13–23. (doi:10.1111/j.0014-3820.2006.tb01077.x)
 14. Ganusov V V, Antia R. 2006 Imperfect vaccines and the evolution of pathogens causing acute in. *Evolution (N. Y.)*. **60**, 957–969. (doi:10.1111/j.0014-3820.2006.tb01174.x)
 15. André J-B, Ferdy J-B, Godelle B. 2003 Within-host parasite dynamics, emerging trade-off, and evolution of virulence with immune system. *Evolution (N. Y.)*. **57**, 1489–1497. (doi:10.1111/j.0014-3820.2003.tb00357.x)
 16. Read AF, Baigent SJ, Powers C, Kgosana LB, Blackwell L, Smith LP, Kennedy DA, Walkden-Brown SW, Nair VK. 2015 Imperfect vaccination can enhance the transmission of highly virulent pathogens. *PLoS Biol.* **13**, 1–18. (doi:10.1371/journal.pbio.1002198)
 17. Fleming-Davies AE, Williams PD, Dhondt AA, Dobson AP, Hochachka WM, Leon AE, Ley DH, Osnas EE, Hawley DM. 2018 Incomplete host immunity favors the evolution of virulence in an emergent pathogen. *Science* **359**, 1030–1033. (doi:10.1126/SCIENCE.AAO2140)
 18. Gandon S. 2018 Evolutionary epidemiology theory of vaccination. In *Pertussis*, pp. 133–143. Oxford University Press. (doi:10.1093/oso/9780198811879.003.0008)
 19. Mattoo et al. 2005 Clinical Manifestations of Respiratory Infections Due to Bordetella

- pertussis and Other *Bordetella* Subspecies Molecular Pathogenesis , Epidemiology , and Clinical Manifestations of Respiratory Infections Due to *Bordetella pertussis* and Other *Bordetella* Su. *Clin. Microbiol. Rev.* **18**, 326–382. (doi:10.1128/CMR.18.2.326)
20. Warfel JM, Merkel TJ. 2014 Reply to Domenech de Celles et al.: Infection and transmission of pertussis in the baboon model. *Proc. Natl. Acad. Sci.* **111**, E718–E718. (doi:10.1073/pnas.1324074111)
 21. Warfel JM, Zimmerman LI, Merkel TJ. 2016 Comparison of three whole-cell pertussis vaccines in the baboon model of pertussis. *Clin. Vaccine Immunol.* **23**, 47–54. (doi:10.1128/CVI.00449-15)
 22. Gill C, Rohani P, Thea DM. 2017 The relationship between mucosal immunity, nasopharyngeal carriage, asymptomatic transmission and the resurgence of *Bordetella pertussis*. *Fl1000Research* **6**, 1568. (doi:10.12688/fl1000research.11654.1)
 23. Rohani P, Earn DJD, Grenfell BT. 2000 Impact of immunisation on pertussis transmission in England and Wales Autoimmune T cells as potential neuroprotective therapy for spinal cord injury. *Lancet* **355**, 285–286.
 24. Taranger J *et al.* 2001 Mass Vaccination of Children with Pertussis Toxoid—Decreased Incidence in Both Vaccinated and Nonvaccinated Persons. *Clin. Infect. Dis.* **33**, 1004–1010. (doi:10.1086/322639)
 25. Sheridan SL, Ware RS, Grimwood K, Lambert SB. 2012 Number and Order of Whole Cell Pertussis Vaccines in Infancy and Disease Protection. *JAMA* **308**, 454–456.
 26. Liko J, Robison SG, Cieslak PR. 2013 Priming with Whole-Cell versus Acellular Pertussis Vaccine. *N. Engl. J. Med.* **368**, 581–582. (doi:10.1056/NEJMc1212006)
 27. Metcalf CJE, Farrar J, Cutts FT, Basta NE, Graham AL, Lessler J, Ferguson NM, Burke

- DS, Grenfell BT. 2016 Use of serological surveys to generate key insights into the changing global landscape of infectious disease. *Lancet* **388**, 728–730. (doi:10.1016/j.jconrel.2012.07.004.Enhanced)
28. Ganusov V V, Bergstrom CT, Antia R. 2002 Within-host population dynamics and the evolution of microparasites in a heterogeneous host population. *Evolution (N. Y.)*. **56**, 213–223. (doi:10.1111/j.0014-3820.2002.tb01332.x)
 29. Ganusov V V., Antia R. 2003 Trade-offs and the evolution of virulence of microparasites: Do details matter? *Theor. Popul. Biol.* **64**, 211–220. (doi:10.1016/S0040-5809(03)00063-7)
 30. Ganusov V V., Anita R. 2006 Imperfect Vaccines and the Evolution of Pathogens Causing Acute Infections in Vertebrates. *Evolution (N. Y.)*. **60**, 957–969.
 31. Gomes MGM, Lipsitch M, Wargo AR, Kurath G, Rebelo C, Medley GF, Coutinho A. 2014 A Missing Dimension in Measures of Vaccination Impacts. *PLoS Pathog.* **10**, e1003849. (doi:10.1371/journal.ppat.1003849)
 32. Greenwood, Yule GU. 1915 The statistics of anti-typhoid and anti-cholera inoculations, and the interpretation of such statistics in general. *Proc. R. Soc. Med.* **8**, 113–194.
 33. Longini IM, Halloran ME. 2006 A Frailty Mixture Model for Estimating Vaccine Efficacy. *Appl. Stat.* **45**, 165. (doi:10.2307/2986152)
 34. Langwig KE *et al.* 2017 Vaccine effects on heterogeneity in susceptibility and implications for population health management. *MBio* **8**, 1–13. (doi:10.1128/mBio.00796-17)
 35. Kermack WO, McKendrick AG. 1927 A Contribution to the Mathematical Theory of Epidemics. *Proc. R. Soc. A Math. Phys. Eng. Sci.* **115**, 700–721.

- (doi:10.1098/rspa.1927.0118)
36. Brännström Å, Johansson J, von Festenberg N. 2013 The Hitchhiker's Guide to Adaptive Dynamics. *Games* (doi:10.3390/g4030304)
 37. Maynard Smith J, Price GR. 1973 The logic of animal conflict. *Nature* **246**, 15–18. (doi:10.1038/246015a0)
 38. Lion S, Metz JAJ. 2018 Beyond R0 Maximisation: On Pathogen Evolution and Environmental Dimensions. *Trends Ecol. Evol.* (doi:10.1016/j.tree.2018.02.004)
 39. Diekmann O, Heesterbeek JAP, Metz JAJ. 1990 On the definition and the computation of the basic reproduction ratio R_0 in models for infectious diseases in heterogeneous populations. *J. Math. Biol. Math. Biol.* **28**, 365–382.
 40. Eshel I. 1983 Evolutionary and continuous stability. *J. Theor. Biol.* **103**, 99–111. (doi:http://dx.doi.org/10.1016/0022-5193(83)90201-1)
 41. R Core Team. 2018 R: A language and environment for statistical computing.
 42. Soetaert K, Petzoldt T, Setzer RW. 2010 Package deSolve : Solving Initial Value Differential Equations in R. *J. Stat. Softw.* **33**, 1–25. (doi:10.18637/jss.v033.i09)
 43. Schmidtke AJ, Boney KO, Martin SW, Skoff TH, Tondella ML, Tatti KM. 2012 Population Diversity among *Bordetella pertussis* Isolates, United States, 1935–2009. **18**, 1991–1996.
 44. Queenan AM, Cassiday PK, Evangelista A. 2013 Pertactin-Negative Variants of *Bordetella pertussis* in the United States. *N. Engl. J. Med.* **368**, 583–584. (doi:10.1056/nejmc1209369)
 45. Mooi FR *et al.* 2009 *Bordetella pertussis* strains with increased toxin production associated with pertussis resurgence. *Emerg. Infect. Dis.* **15**, 1206–1213.

(doi:10.3201/eid1508.081511)

46. Rohani P, Drake JM. 2011 The decline and resurgence of pertussis in the US. *Epidemics* **3**, 183–188. (doi:10.1016/j.epidem.2011.10.001)
47. Althouse BM, Scarpino S V. 2015 Asymptomatic transmission and the resurgence of *Bordetella pertussis*. *BMC Med.* **13**, 1–12. (doi:10.1186/s12916-015-0382-8)
48. Jackson DW, Rohani P. 2014 Perplexities of pertussis: Recent global epidemiological trends and their potential causes. *Epidemiol. Infect.* **142**, 672–684.
(doi:10.1017/S0950268812003093)
49. World Health Organization. 2015 Weekly epidemiological record: position paper on pertussis vaccines. *Wkly. Epidemiol. Rec.* **90**, 433–60. (doi:10.1371/jour)
50. World Health Organization. 2018 WHO Mortality Database. *Heal. Stat. Heal. Inf. Syst.*
(doi:10.1002/psp)

Chapter II: Disease and healthcare burden of COVID-19 in the United States

Foreword

This chapter was published as a Letter in Nature Medicine in June of 2020:

Miller, I.F., Becker, A.D., Grenfell, B.T., and Metcalf, C. J. E. Disease and healthcare burden of COVID-19 in the United States. *Nat Med* 26, 1212–1217 (2020). <https://doi.org/10.1038/s41591-020-0952-y>

Abstract

As of April 24, 2020, the SARS-CoV-2 epidemic has resulted in over 830,000 confirmed infections in the United States of America [1]. The incidence of COVID-19, the disease associated with this novel coronavirus, continues to rise. The epidemic threatens to overwhelm healthcare systems and identifying those regions where the disease burden is likely to be high relative to the rest of the country is critical for enabling prudent and effective distribution of emergency medical care and public health resources. Globally, risk of severe outcomes associated with COVID-19 has consistently been observed to increase with age [2,3]. We used age-specific mortality patterns in tandem with demographic data to map projections of the cumulative case burden of COVID-19 and the subsequent burden on healthcare resources . The analysis was performed at the county level across the United States assuming a scenario in which 20% of the population of each county acquires infection. We identified counties that will likely be consistently, heavily affected relative to the rest of the country across a range of assumptions about transmission patterns, such as the basic reproductive rate, contact patterns, and the efficacy of quarantine We observed a general pattern that per capita disease burden and relative healthcare system demand may be highest away from major population centers. These findings highlight the importance of ensuring equitable and adequate allocation of medical care and public health resources to communities outside of major urban areas.

Introduction

SARS-CoV-2 was first identified in December 2019 in Wuhan, China [4], and the first infection detected in the United States on January 7th 2020 [5]. The virus is both highly transmissible and virulent. Estimates of the basic reproductive number, R_0 , range from 1.4 to 6.49 [6], with an estimated overall case fatality rate of 1.4% [7], which is highly varied across age classes. As of April 24, 2020, SARS-Cov-2 over 2,626,000 confirmed cases and over 181,000 deaths had been recorded globally. Currently, the cumulative reported incidence of COVID-19 in the U.S. is the highest in the world [1].

As the COVID-19 epidemic expands within the U.S., a central focus of public health efforts will be limiting fatalities. A key driver of this outcome will be keeping the case burden of patients with COVID-19 within the treatment capacity of the healthcare system. If the medical system is overwhelmed, the standard of care for all individuals seeking medical care could be reduced, thereby exacerbating negative health outcomes [8]. Critically ill patients with COVID-19 might fare particularly poorly. High mortality rates within this group will likely be further compounded by shortages of intensive care facilities and/or access to mechanical ventilation equipment [9]. Patients without COVID-19, who require care for other conditions will also be affected by the health system's inability to meet their needs.

Effective allocation of limited medical resources, such as healthcare workers, protective equipment and ventilators, is required to reduce the likelihood of the healthcare system being overwhelmed. However, in order to achieve this, information on the distribution of the burden of disease and how that burden aligns with healthcare system capacity is required.

Several factors likely contribute to the heterogeneous distribution of COVID-19 burden across the U.S. The first of these is demography. Incidence of COVID-19 consistently rises with

increasing age [2,3] (noting that incidence observed from diagnostic testing does not necessarily reflect total infections). This age-dependent pattern of infection seems to be largely driven by differences in susceptibility and symptomatic infection rates between age classes rather than differences in transmission potential [3,10]. Rates of hospitalization and intensive care unit admission are also higher in individuals aged over 60 years than in younger age classes [11]. Thus, variation in age structure between counties could lead to differences in the per capita burden of disease between regions. Access to health care could also affect the distribution of COVID-19 burden. Many rural areas of the U.S. might have insufficient or no resources to provide acute or critical care. Residents of such areas could therefore be at increased risk for insufficient treatment. Finally, limited healthcare system capacity in rural areas could lead to an unexpected influx of cases to hospitals in more densely populated regions.

The temporal distribution of COVID-19 spread could also contribute to heterogeneity in disease burden across the USA. The magnitude and timing of the epidemic peak, for example, determines the minimum healthcare system capacity needed to provide adequate care. However, obtaining accurate predictions of the epidemic peak is often challenging in emerging outbreaks, due to limited and often unreliable data on incidence, as well as the challenges associated with modelling the effects of rapidly deployed and changing mitigation efforts. County-level variability in testing standards and efforts [12,13], non-pharmaceutical interventions (NPIs) such as social distancing [14], and outbreak onset [15] and a lack of serological data also limit efforts to accurately model epidemic trajectories beyond several weeks. By contrast, projections of cumulative disease burden are less hindered by these challenges as they are not aimed at describing an epidemic time course. Although, such projections miss the nuance of the intensity and timing of outbreaks, their estimates of the spatial footprint of disease burden contains core

information relevant to informing resource distribution. Comparing the expected cumulative number of critical and severe infections against healthcare resources in each county in the U.S. allows for the identification of regions which may experience particularly high disease burden. Furthermore, analyzing simulations of multiple transmission scenarios (e.g., different contact patterns) allows for possible identification of those regions with consistently high disease burdens without needing to forecast an exact epidemic trajectory.

Here, we project the cumulative case burden (case numbers) and cumulative per capita burden (cases per person) of severe and critical COVID-19 cases in each county within the U.S. by combining demographic data and age-specific risk factors under the assumption that 20% of the population becomes infected. We calculate the cumulative healthcare system burden using case to bed ratio that each county could experience as its own residents (and those from nearby counties with limited or non-existent medical resources) seek care. We repeat this analysis for a range of transmission scenarios, map expected burden of COVID-19 for each scenario, and identify the regions consistently expected to experience the highest cumulative burden of disease. A summary of the main findings, limitations, and policy implications of this study is given in Table 1.

Background	The now widely prevalent novel coronavirus SARS-CoV-2 poses a grave public health threat in the United States, particularly in areas where cases could overwhelm healthcare systems. Allocating emergency medical and public health resources can help to mitigate this problem, but this efficiently requires identifying regions that are severely affected.
Main findings and limitations	Leveraging the relationship between age and COVID-19 disease severity, we projected the cumulative disease burden for each of 3,142 counties and county equivalents in the U.S. using a modified SEIR model. Comparing these burdens to hospital capacity, we identified counties that will likely be consistently, heavily affected relative to the rest of the country, and found that the per capita burden of disease and the relative strain on the healthcare system could be higher in rural areas. To circumvent uncertainty surrounding the time course of the epidemic, we projected long-term cumulative burden of COVID-19 rather than peak burden. These projections ignore the temporal component of disease spread, and assume that cumulative incidence is identical among counties. Due to these limitations, our results should be interpreted as an approximation of the spatial distribution of COVID-19 burden rather than as a set of epidemiological forecasts.
Policy implications	Our findings highlight the importance of ensuring equitable and adequate distribution of medical care and public health resources to urban and rural areas to reducing the total mortality associated with COVID-19.

Table 1: Policy Summary

Results

We developed a modified age-stratified Susceptible-Exposed-Infected-Recovered (SEIR) epidemic model (based on the model of Davies et al. [3]) to project the number of COVID-19 cases for all counties (and county equivalents such as independent cities) in the U.S. In this model, susceptible individuals (S) become infected in a density-dependent fashion and enter the exposed (E) class, before eventually becoming either asymptotically infected (I_A) or mildly symptomatic (but not yet clinically presenting) (I_P). Following published estimates [3], we assume relative susceptibility to infection and the fraction of individuals who become mildly symptomatic rather than asymptotically infected are higher in older age classes than in younger age classes. Individuals in the I_P class eventually become fully symptomatic (I_C). Asymptomatic and symptomatic individuals recover with immunity to classes R_A and R_S , respectively. All individuals in the infected classes (I_A , I_P , I_C) are infectious; however, our model assumes that the relative infectiousness of asymptomatic individuals is scaled by a factor b_a , and the relative infectiousness of fully symptomatic individuals is scaled by a factor b_C to account for the effects of case isolation and quarantine. Mixing between individuals of different age classes is determined by a parameter θ . For $\theta = 1$, mixing patterns reflect empirically measured rates for the U.K.[16]. For $\theta = 0$, mixing patterns are homogeneous. For $0 < \theta < 1$, mixing patterns are intermediate. This model aims to specifically project the age distribution of cases over a wide variety of transmission scenarios, and is not intended to produce epidemiological forecasts. As such, we include epidemiological details that could result in differences in disease burden between age classes, such as age-specific mixing patterns and rates of symptom presentation. However, we do not vary the components of our model linked to interventions (e.g. transmission rate, mixing patterns) over time or by location.

We investigated a scenario in which 20% of the population in each county becomes infected. A 20% cumulative infection rate represents a pessimistic scenario over the next several months, but perhaps this will be an optimistic scenario beyond that time frame [17]. A 20% cumulative infection rate is independent of R_0 and is not equivalent to 20% of the herd immunity threshold. We intentionally ignored spatial variation in the progression of the epidemic to simplify comparisons of disease burden between regions.

As we aim to provide general estimates of relative distribution of disease burden rather than make precise predictions of case load over time, we sought to identify patterns of disease burden that are robust to different assumptions about the dynamics of epidemiological spread. Accordingly, we varied our assumptions about the overall transmissibility of COVID-19, age-structure of contact patterns, and the contributions of fully symptomatic individuals to transmission. For each set of assumptions, we simulated our model for each county in the U.S. using demography data from the 2018 American Community Survey [18]. We then extracted the number of individuals in each age class who had become symptomatically infected by the time the cumulative population infection rate had reached 20%. We present detailed results for the most optimistic scenario and most pessimistic scenario. In the optimistic scenario (transmission, $R_0 = 2$, relative infectivity of fully symptomatic individuals, $b_C = 0.1$, mixing structure, $\Theta = 1$, see *Methods*) transmission is slow, fully symptomatic individuals are effectively quarantined, and mixing patterns exhibit a strong age structure, potentially decreasing transmission from asymptotically infected (and thus non-quarantined) individuals in less vulnerable age classes (such as children) to individuals in more vulnerable age classes (such as the elderly). By contrast, the pessimistic scenario ($R_0 = 5$, $\Theta = 0$, $b_C = 1$) is characterized by high transmission, well-mixed

contact patterns, and ineffective quarantine. Results for 25 alternate combinations of R_0 , Θ , and b_C are summarized in Extended Data Figures 1-4.

To evaluate the sensitivity of our results to effects of crowding on transmission and epidemic size, we also investigated alternative scenarios in which R_0 increases as a linear function of urban population, from $R_0 = 2$ in counties with 0% of residents living in urban areas, to $R_{0_{max}}$ in counties with 100% of residents living in urban areas. In an optimistic scenario we set $R_{0_{max}}$ to 3 (other parameters: $\Theta = 1$, $b_C = 0.1$), and in a pessimistic scenario we set $R_{0_{max}}$ to 5 (other parameters: $\Theta = 0$, $b_C = 1$). Disease burden in each county was calculated when the cumulative number of infections reached 20% of the herd immunity threshold multiplied by the population size, rather than 20% of the population size (see Methods). The relationship between crowding and R_0 has not been definitively established, and as such these results should be interpreted cautiously.

Using our projections of cumulative symptomatic infections, we further estimated the number of severe cases (i.e., requiring hospitalization) and critical cases (i.e., requiring intensive care) using published rates of these outcomes for various age classes [11]. In all transmission scenarios, the areas with high relative burdens of hospitalizations and intensive care unit (ICU) admissions generally had large populations (Fig. 1 A, D, H, Fig. 2 A, D, Fig. 3 A, D). However, we observed the opposite pattern for the per capita burden of hospitalizations and ICU admissions, which were distributed heterogeneously and were higher in rural areas than in major population centers (Fig. 1 C, F, J). Due to the positive correlation between age and disease severity, the areas with the highest per capita burden were those with the highest percentages of individuals over the age of 60 years. (Figure 1 B, E, I, Figure 2 B, E, and Figure 3 B, E). Although more elderly age classes were disproportionately affected in the pessimistic

transmission scenario (Figure 2 G, Figure 3 G), the sets of counties with very high projected burdens of per capita hospitalizations and ICU admissions remained similar across different transmission scenarios. Indeed, of the 315 counties at or above the 90% quantile of per capita hospitalization in the optimistic transmission scenario (Figure 1 legend), 308 counties were also at or above this quantile in the pessimistic scenario. The median percentage of people residing in rural areas among these 308 counties was 100%, which is significantly greater than the median of all counties (57.54%, Mann-Whitney $U=696348$, $n_1=308$, $n_2=3142$, two-sided $p<2.2*10^{-16}$). Of the 315 counties at or above the 90% quantile of per capita ICU admissions in the optimistic transmission scenario, 313 were also at or above this quantile in the pessimistic scenario. Again, the median percentage of people residing in rural areas among these 313 counties was 100%, significantly greater than the median of all counties (Mann-Whitney $U=725670$, $n_1=313$, $n_2=3142$, two-sided $p<2.2*10^{-16}$).

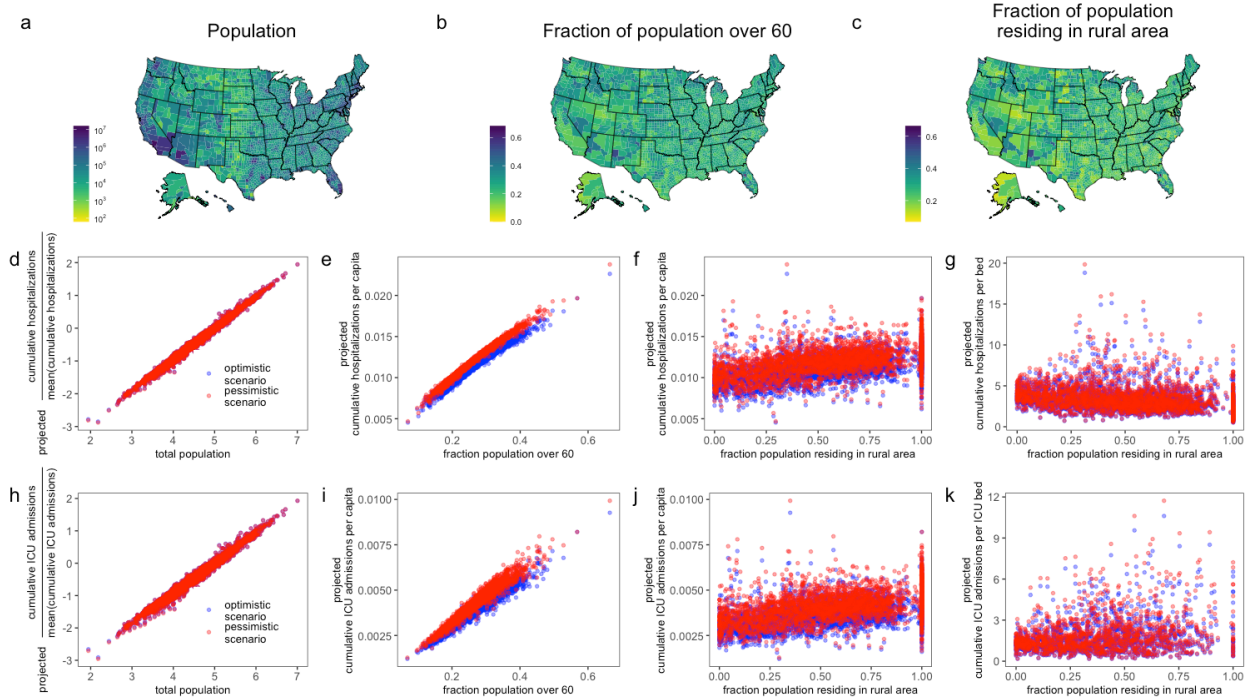


Figure 1: Population characteristics of the U.S. and their relationships with disease burden

A Population of each county. B Fraction of individuals within each county over the age of 60 years. C Fraction of the population of each county classified as living in a rural area according to the 2010 U.S. Census[19]. D-K The relationship between population characteristics (x-axes) and metrics of disease burden (y-axes) for the optimistic transmission scenario (blue) and the pessimistic scenario (red).

Next, we evaluated how projected case burdens aligned with healthcare system capacity. We obtained data on the number of hospital beds and ICU beds in each county from the American Hospital Association 2018 annual survey [20]. We distributed cases to healthcare systems within and outside of their county of origin based on an allocation algorithm (see *Methods*). This algorithm distributes severe and critical cases based on relative distance and the relative capacity of healthcare systems to provide care (quantified as the number of hospital beds and ICU beds, respectively). The majority of cases originating from within a county with substantial medical resources stays within that county. Most severe and critical cases originating

from within a county with few hospitals or ICU beds are allocated to nearby counties with greater care capacity. All severe or critical cases originating in a county that lacks the capacity to provide appropriate care entirely are distributed to nearby counties.

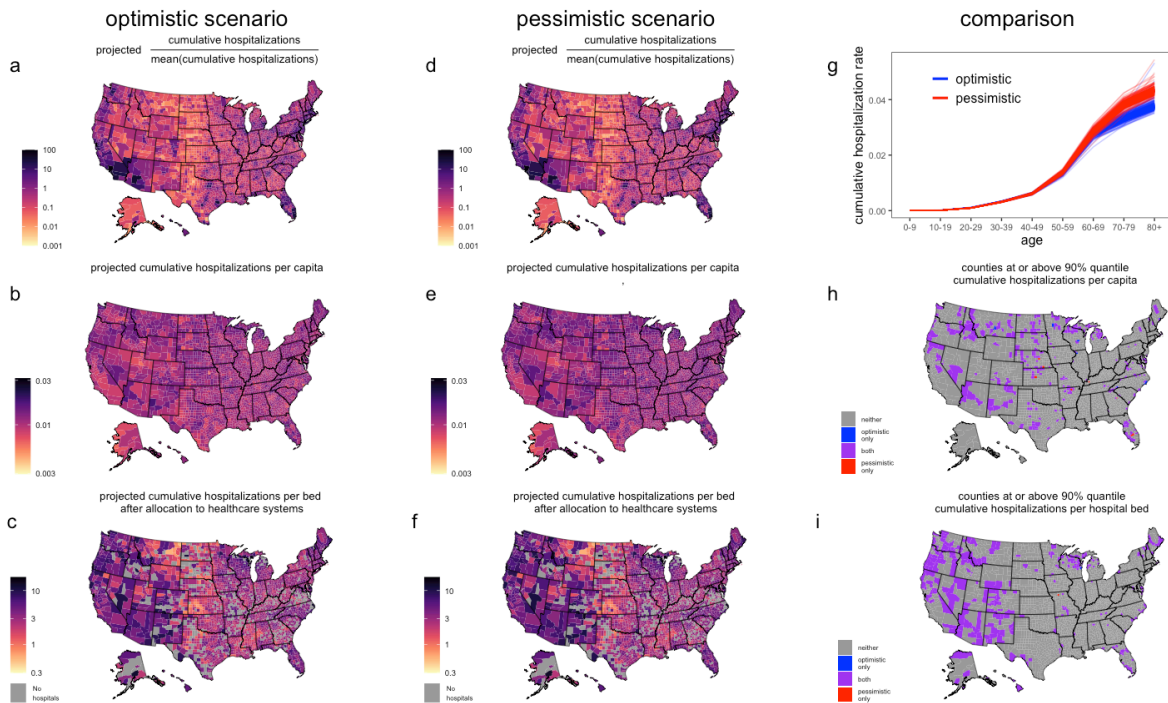


Figure 2: Projected cumulative burden of hospitalizations in the U.S.

A, B, and C Optimistic scenario. Panels D, E, and F Pessimistic scenario. A and D Relative number of hospitalizations in each county. B and E Number of projected hospitalizations per capita in each county. In A, B, D, and E cases have not yet been allocated to healthcare systems. C and F Cumulative number of hospitalizations per hospital bed after cases have been allocated to healthcare systems. G Cumulative fraction of each age class hospitalized in each transmission scenario. Each of the 315 lines for each transmission scenario represents a different county. H and I Counties estimated to be in the 90% quantile of hospitalizations per capita and hospitalizations per hospital bed (after case allocation). Colors in H and I indicate whether the counties were estimated to be in the 90% quantile in the optimistic scenario, the pessimistic scenario, both, or neither.

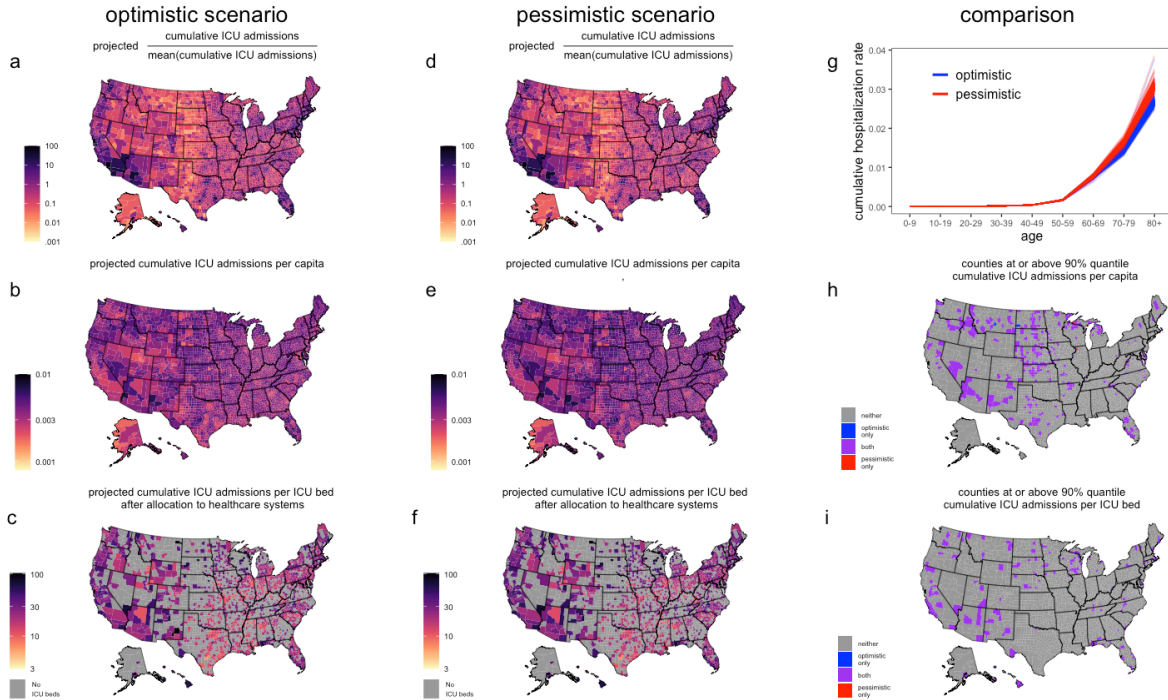


Figure 3: Projected cumulative burden of ICU admissions in the U.S.

A, B, and C Optimistic scenario. Panels D, E, and F Pessimistic scenario. A and D Relative number of ICU admissions in each county. B and E The number of projected ICU admissions per capita in each county. In A, B, D, and E cases have not yet been allocated to healthcare systems. C and F Cumulative number of ICU admissions per ICU bed after cases have been allocated to healthcare systems. G Cumulative fraction of each age class requiring ICU admission in each transmission scenario. Each of the 315 lines for each transmission scenario represents a different county. H and I Counties estimated to be in the 90% quantile of ICU admissions per capita and ICU admissions per ICU bed (after case allocation). Colors in H and I indicate whether the counties were estimated to be in the 90% quantile in the optimistic scenario, the pessimistic scenario, both, or neither.

The maps of relative hospitalizations per bed (Figure 2 C, F) and relative ICU admissions per bed (Figure 3 C, F) indicate counties expected to experience a higher burden of disease relative to medical resources. The burden of cases relative to hospital and ICU beds was

generally highest away from urban centers in counties with substantial rural populations (Extended Data Fig. 5 D,H). Several regions have a high concentration of counties with high burden, including much of the western United states, the northern Midwest, Florida, and northern New England. These patterns are robust to assumptions about transmission rates and age-specific mixing patterns. The optimistic and pessimistic transmission scenarios each identified 248 counties as being at or above the 90% quantile of cumulative hospitalizations per hospital bed; 247 counties were identified in both transmission scenarios. The median percentage of people residing in rural areas among these 247 counties (38.97%) is lower than the median for all U.S. counties with hospital beds (51.82%, Mann-Whitney $U=246652$, $n_1=247$, $n_2=2478$, two-sided $p=4.64 \times 10^{-7}$). Nevertheless these data indicate that the healthcare system burden is not concentrated in urban centers. In the case of ICU admissions per bed, all of the 136 counties identified as being at or above the 90% quantile were the same for both transmission scenarios. These 136 counties (median percentage of residents living in rural areas = 31.11%) were not identified as being more rural than all U.S. counties with ICU beds (median percentage of residents living in rural areas = 36.21%, Mann-Whitney $U=85746$, $n_1=136$, $n_2=1353$, two-sided $p=0.19$), but again, a pattern of healthcare system burden not being concentrated in urban areas emerges.

For analyses where R_0 varied as a function of the percentage of population residing in urban areas, the per capita and per hospital and ICU bed burdens of disease were not generally higher in rural areas, but rather distributed heterogeneously across urban and rural areas (Extended Data Fig. 6). Counties at or above the 90% quantile for various metrics of disease burden were less rural than comparable counties, but were not heavily concentrated in the urban end of the urban-rural distribution (Extended Data Figs. 9-10). Otherwise, results from these

analyses (Extended Data Figs. 6-10) largely agreed with those presented above, indicating that our finding that disease burden is not expected to be concentrated only in urban areas is robust to assumptions about the effects of crowding on transmission patterns and epidemic size.

Discussion

Even with unprecedented efforts to rapidly develop a vaccine [21], a pharmaceutical intervention against COVID-19 is unlikely to be available in the near future. SARS-Cov-2 transmission is expected to continue over the coming months and will likely affect every locality in the U.S. We aimed to identify counties that consistently emerge as being likely to experience a large burden of disease on their population and healthcare systems (across a range of assumptions about transmission patterns). We identified several regions in need of additional support, including much of the western portion of the country, the northern Midwest, Florida, and northern New England. At a fine geographical scale, our results suggest that considerable rural-urban inequities exist, with the per capita burden of disease being higher away from major population centers.

Before even considering the increased case burden these more rural places are projected to experience relative to the rest of the U.S., it is evident that hospitals, and to a greater extent, hospitals with the capacity to provide intensive care, are unevenly distributed. Many regions have limited, or no, facilities equipped to provide the type of acute or critical care required to treat COVID-19¹⁹. Case fatality rates in these regions could rise above the national average if people are unable to access care. Bolstering the capacity of rural health systems, ensuring equitable access to care, and implementing public health measures such as testing and contact tracing in both urban and rural areas should be central goals of COVID-19 management strategies in the USA. While the healthcare systems of major population centers were not

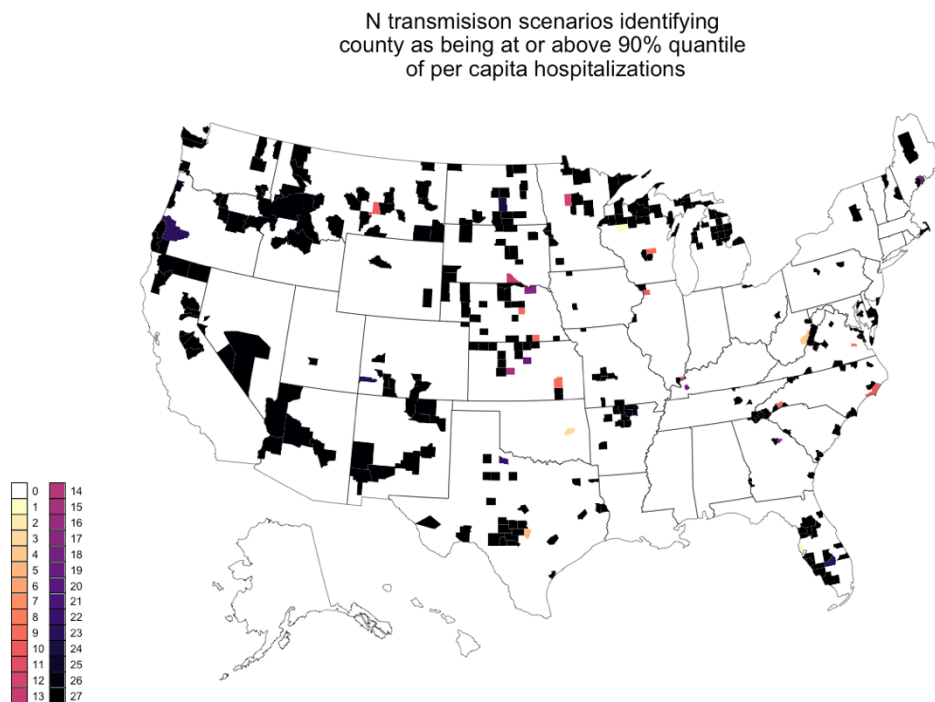
identified as weak spots in our analysis, they do service a much larger number of people. Given the consequences of their potential failure, they should remain a priority for response efforts.

Our findings are robust to different assumptions about transmission patterns. However, it is imperative that they be interpreted in the context of our methodology. We were deliberately conservative in not considering the impact of potential therapeutics and vaccines. Our results only underscore the urgency of developing these interventions. Likewise, we did not consider the impact of other NPIs such as social distancing. Our findings point to the importance of implementing these measures in urban and rural regions. We specifically did not attempt to predict the epidemic peak timing or magnitude. Given the time-invariant scenario we model (i.e., 20% of the population acquires infection), it is likely that our projections will not precisely match future observed patterns of disease burden in the short term as many regions are still in the early phases of their epidemics, or in the long term, as the timing, extent, and efficacy of interventions will vary between regions. However, our results provide an approximation of the expected patterns of burden rooted in basic features of demography and health system capacity. Notably, we did not consider how other factors linked with an increased risk of severe disease, such as comorbidities [22] (e.g. hypertension, pulmonary disease), or decreased access to medical care, such as uninsurance rate and socioeconomic status [23], might exacerbate disease burden in certain regions. Incorporating such factors into mathematical models and their forecasts is an essential area of future research, and could reveal additional ‘hotspots’ of disease burden that were not identified in our analyses, which considered the role of demography alone. Future work should also seek to identify if and where disease burden is disproportionately high in certain racial or minority groups. Finally, we urge public health officials using our results to carefully consider location-specific details and nuances not explicitly included in our analyses

when planning their response, and to focus on patterns of relative burdens rather than projections for individual counties.

In conclusion, we have identified areas in the U.S. expected to be particularly heavily affected by COVID-19. Our findings suggest that ensuring equitable allocation of medical care and public health resources to communities away from major population centers will be crucial as the US attempts to mitigate the consequences of the ongoing COVID-19 epidemic.

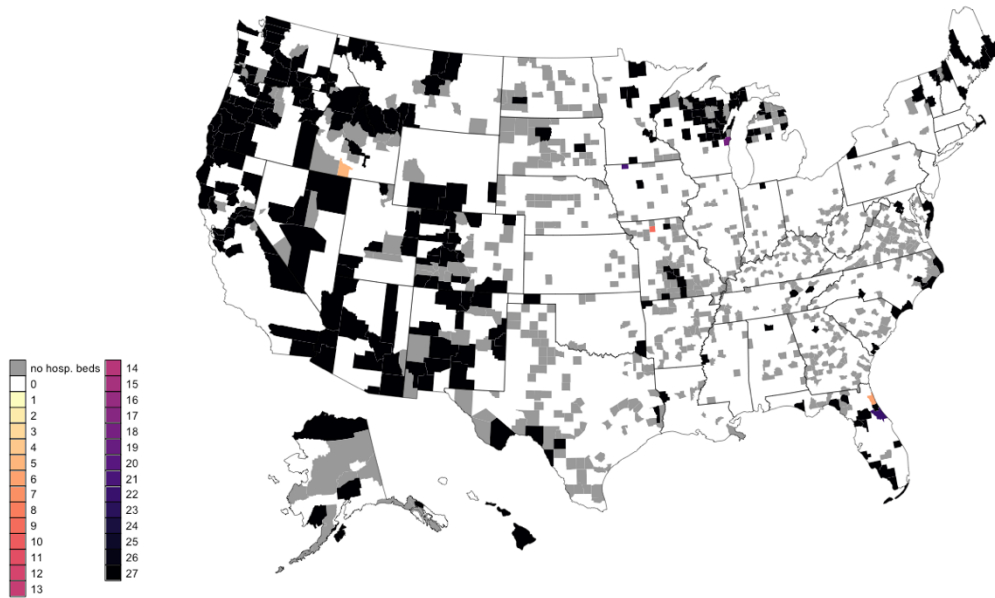
Extended data figures



Extended Data Figure 1: Counties at or above 90% quantile of per capita hospitalizations

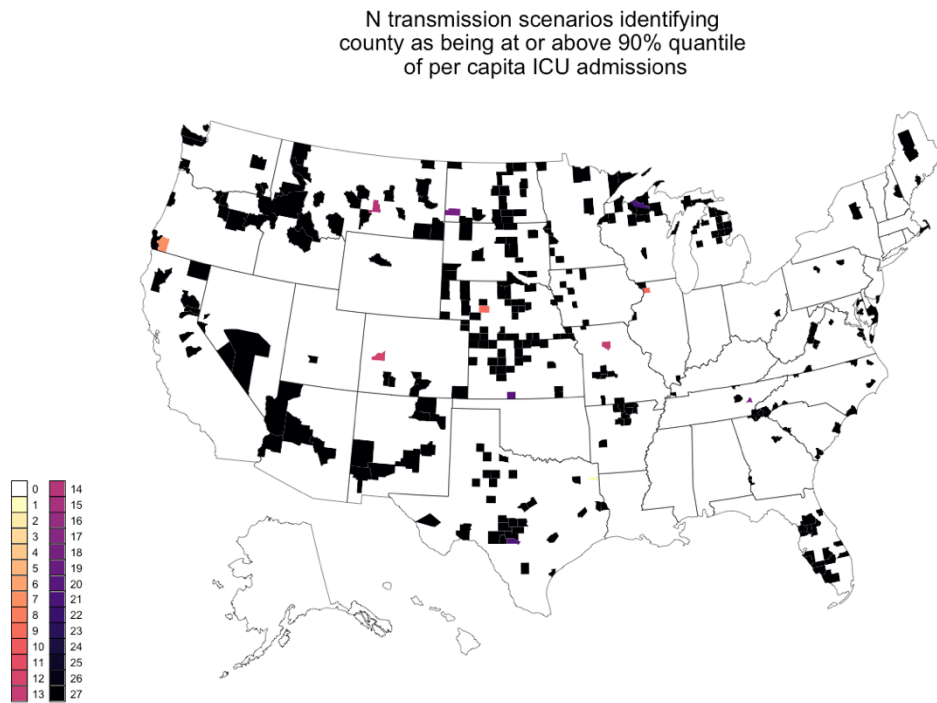
Colors indicate the number of transmission scenarios in which each county was identified as being at or above the 90% quantile of per capita hospitalizations.

N transmission scenarios identifying
county as being at or above 90% quantile
of hospitalizations per hospital bed



Extended Data Figure 2: Counties at or above 90% quantile of hospitalizations per hospital bed

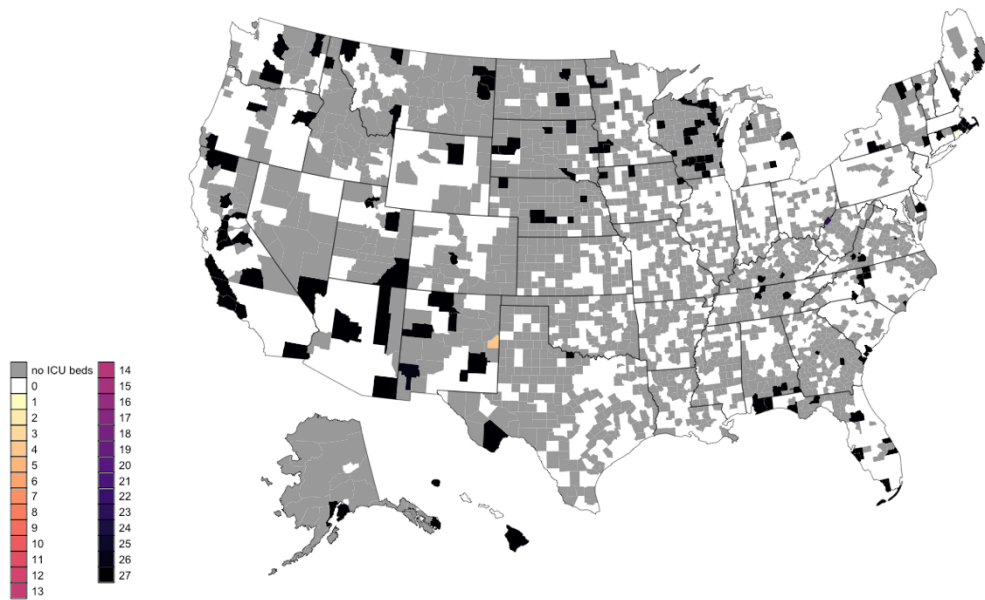
Colors indicate the number of transmission scenarios in which each county was identified as being at or above the 90% quantile of hospitalization per hospital bed. Counties without hospital beds are colored grey.



Extended Data Figure 3: Counties at or above 90% quantile of per capita ICU admissions

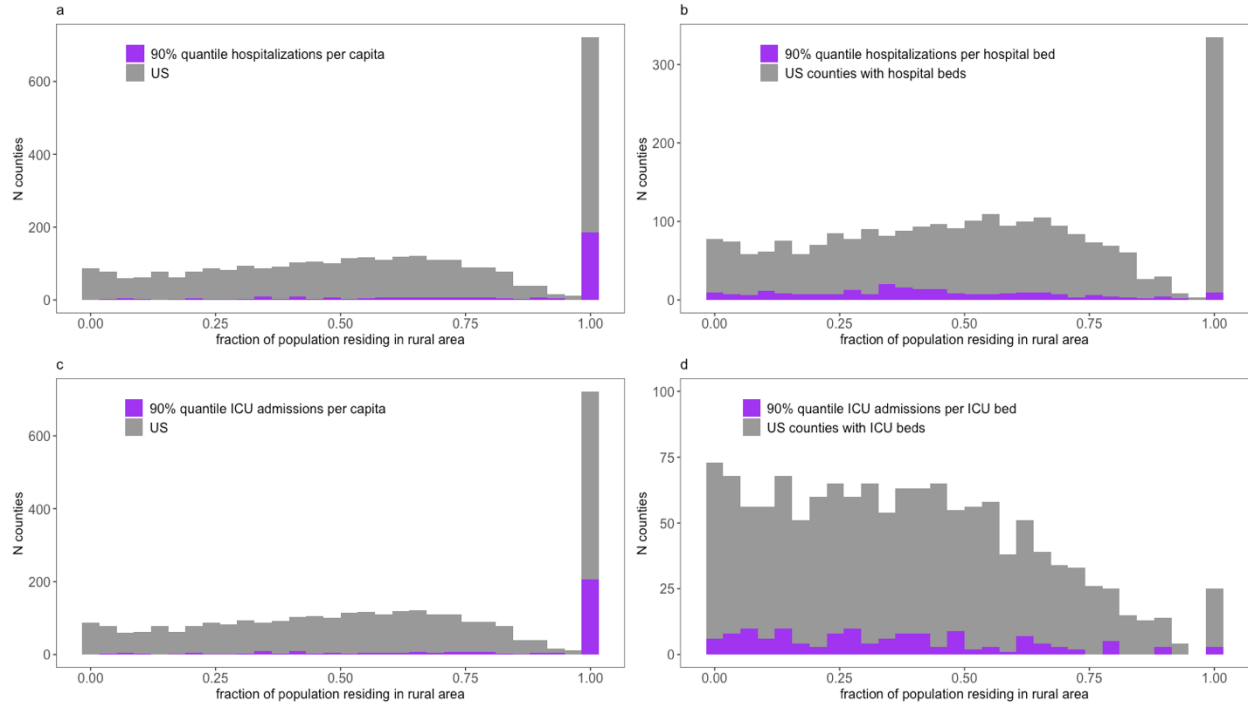
Colors indicate the number of transmission scenarios in which each county was identified as being at or above the 90% quantile of ICU admissions per capita.

N transmission scenarios identifying
county as being at or above 90% quantile
of ICU admissions per ICU bed



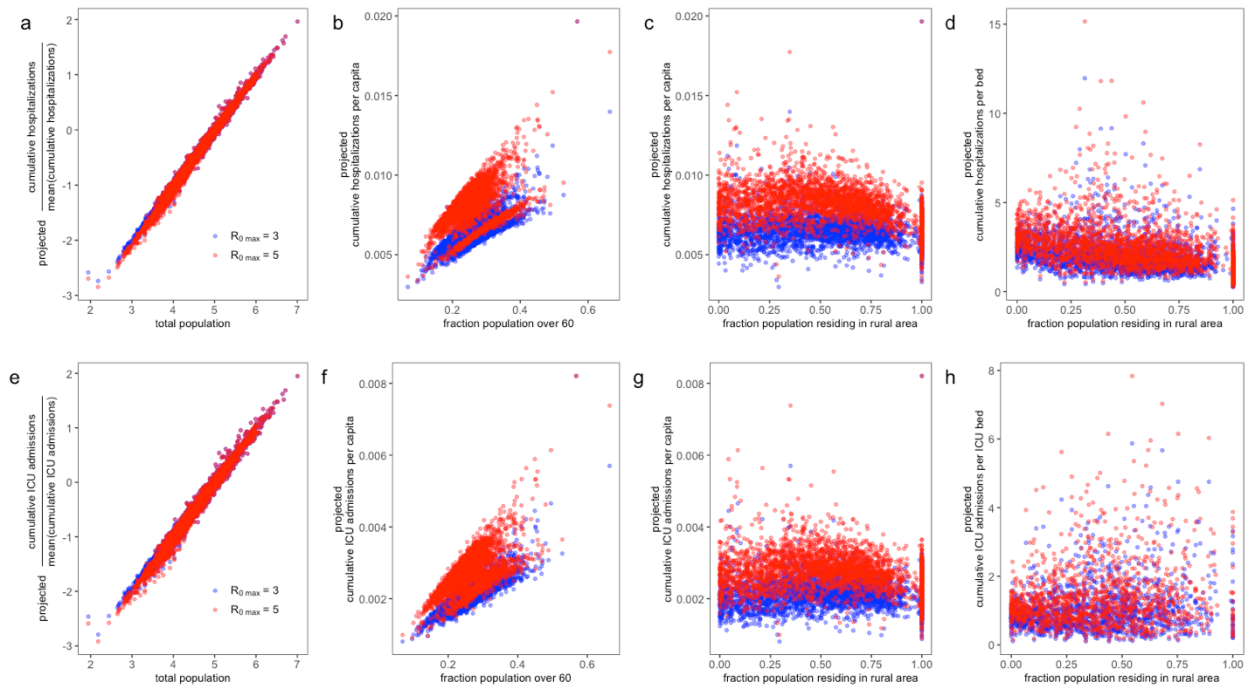
Extended Data Figure 4: Counties at or above 90% quantile of per capita ICU admissions per ICU bed

Colors indicate the number of transmission scenarios in which each county was identified as being at or above the 90% quantile of ICU admissions per ICU bed. Counties without ICU beds are colored grey.



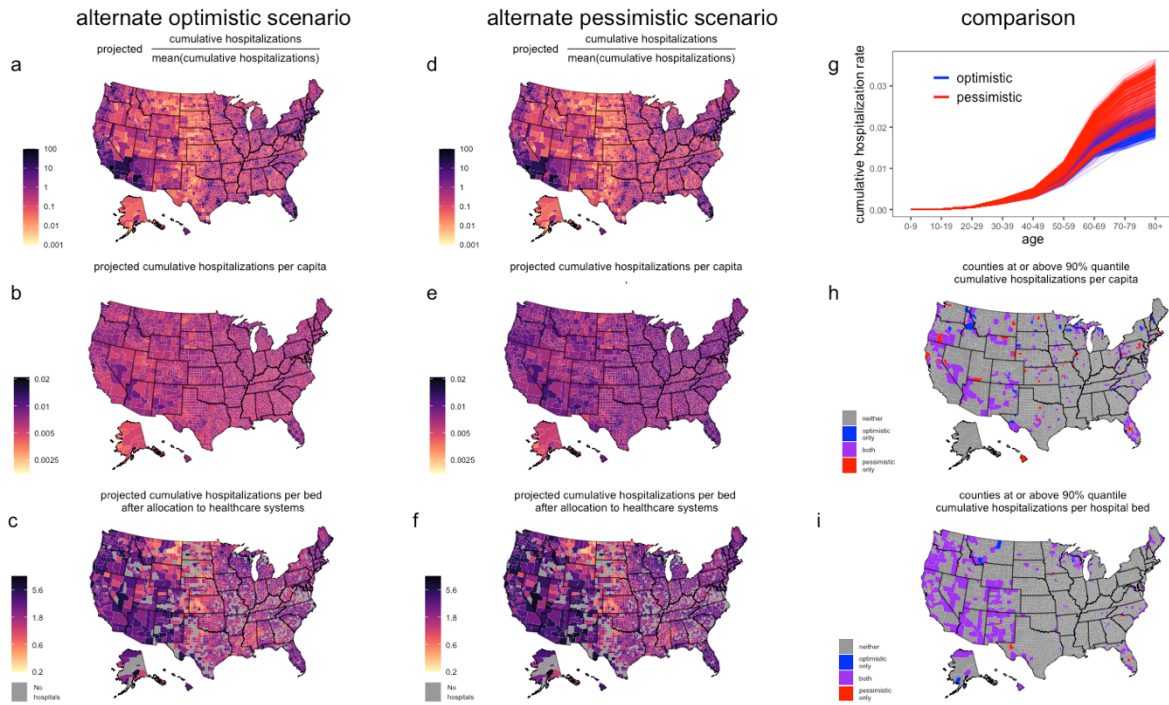
Extended Data Figure 5: The rural-urban distribution of U.S. counties with heavy COVID-19 burden

A Per capita hospitalizations. B Cumulative hospitalizations per hospital bed. C per capita ICU admissions. D Cumulative hospitalizations per ICU bed. Counties identified as being in the 90% quantile for various metrics of disease burden in the optimistic and pessimistic transmission scenarios (purple bars) and comparable counties across the entire US (grey bars).



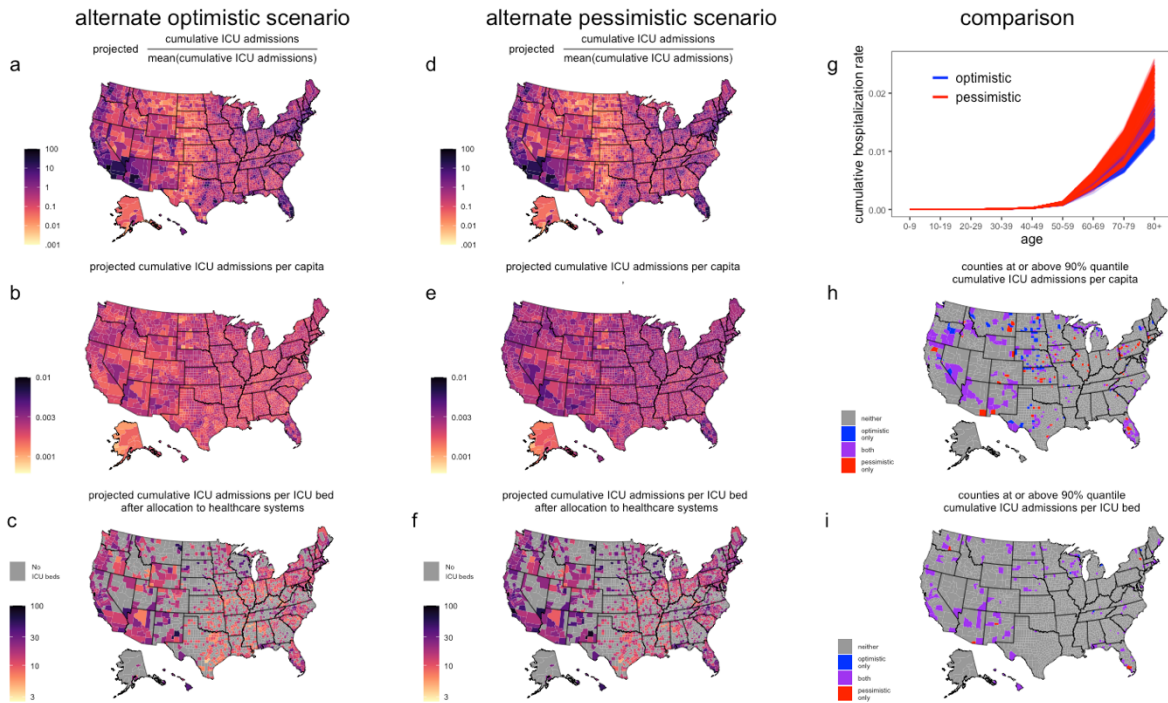
Extended Data Figure 6: Relationships between population characteristics and disease burden for alternative optimistic and pessimistic scenarios

A-H Relationship between population characteristics (x-axes) and metrics of disease burden (y-axes) for the alternative optimistic (blue points) and pessimistic (red points) scenarios in which R_0 increased as a linear function of urban population.



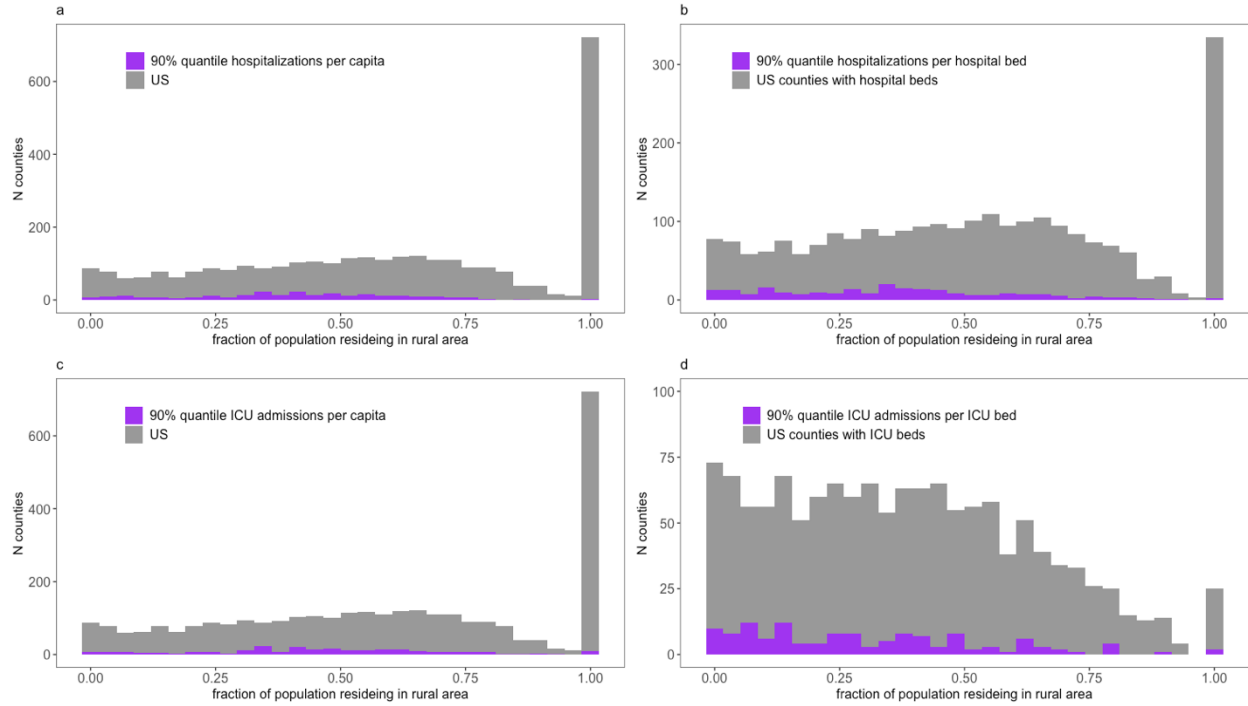
Extended Data Figure 7: Projected cumulative burden of hospitalizations in the U.S. for alternative optimistic and pessimistic scenarios

In the alternative optimistic and pessimistic scenarios, R_0 increased as a linear function of urban population. A, B, and C optimistic scenario. D, E, and F pessimistic scenario. A and D Relative number of hospitalizations in each county. B and E Number of projected hospitalizations per capita in each county. In A, B, D, and E cases have not yet been allocated to healthcare systems. C and F Cumulative number of hospitalizations per hospital bed after cases have been allocated to healthcare systems. G Cumulative fraction of each age class hospitalized in each transmission scenario. Each of the 315 lines for each transmission scenario represents a different county. H and I Counties estimated to be in the 90% quantile of hospitalizations per capita and hospitalizations per hospital bed (after case allocation). Colors in H and I indicate whether the counties were estimated to be in the 90% quantile in the optimistic scenario, the pessimistic scenario, both, or neither. A high-resolution version of this figure is provided in the Supplementary Information.



Extended Data Figure 8: Projected cumulative burden of ICU admissions in the U.S. for alternative optimistic and pessimistic scenarios

In the alternative optimistic and pessimistic scenarios, R_0 increased as a linear function of urban population. A, B, and C Optimistic scenario. D, E, and F Pessimistic scenario. A and D Relative number of ICU admissions in each county. B and E Number of projected ICU admissions per capita in each county. In A, B, D, and E cases have not yet been allocated to healthcare systems. C and F Cumulative number of ICU admissions per ICU bed after cases have been allocated to healthcare systems. G Cumulative fraction of each age class requiring ICU admission in each transmission scenario. Each of the 315 lines for each transmission scenario represents a different county. H and I Counties estimated to be in the 90% quantile of ICU admissions per capita and ICU admissions per ICU bed (after case allocation). Colors in H and I indicate whether the counties were estimated to be in the 90% quantile in the optimistic scenario, the pessimistic scenario, both, or neither. A high-resolution version of this figure is provided in the Supplementary Information.



Extended Data Figure 9: The rural-urban distribution of U.S. counties with heavy COVID-19 burden for alternative optimistic and pessimistic scenarios

In the alternative optimistic and pessimistic scenarios, R_0 increased as a linear function of urban population. A Per capita hospitalizations. B Cumulative hospitalizations per hospital bed. C per capita ICU admissions. D Cumulative hospitalizations per ICU bed. Counties identified as being in the 90% quantile for various metrics of disease burden in the optimistic and pessimistic transmission scenarios (purple bars) and comparable counties across the entire US (grey bars).

Metric	Median percentage of population residing in rural area		Mann-Whitney U	Two-sided p value
	Counties identified at or above 90% quantile	Comparable counties		
Per capita hospitalizations	41.01 (N = 269)	59.54 (N total counties = 3142)	277982	$<2.2 \times 10^{-16}$
Per capita ICU admissions	45.18 (N = 245)	59.54 (N total counties = 3142)	291914	2.35×10^{-10}
Hospitalizations per hospital bed	34.52 (N = 241)	51.82 (N counties with hospital beds = 2478)	201652	$<2.2 \times 10^{-16}$
ICU admissions per ICU bed	27.02 (N = 131)	36.21 (N counties with ICU beds = 1353)	73457	1.2×10^{-3}

Extended Data Figure 10: The comparative rurality of U.S. counties with heavy COVID-19 burden for alternative optimistic and pessimistic scenarios

In the alternative optimistic and pessimistic scenarios, R_0 increased as a linear function of urban population. Mann-Whitney U tests indicated that the sets of counties identified as being heavily burdened in both scenarios were less rural than the set of comparable counties.

Methods

Data

We obtained counts of the number of individuals in 10 year age bins for all U.S. counties (we include non-county federally incorporated places in the set of all counties for the purposes of our analyses) from the 2018 American Community Survey available from the United States Census Bureau [18]. We define the set of age categories as $G=\{0-9, 10-19, \dots, 70-79, 80+\}$. We obtained data on hospital location and bed number from the American Hospital Association 2018 annual survey [20]. We used the calculated total of all beds for each hospital to represent the number of hospital beds, and the number of adult medical/surgical intensive care beds to represent the number of intensive care unit beds. We aggregated hospital and ICU bed data by county in accordance with American Hospital Association data use policy. We obtained the numbers of individuals in each county living in rural and urban areas from the 2010 U.S. census [19].

Mechanistic models

We developed an age stratified mechanistic epidemiological model based on that of Davies et al. [3] that follows a susceptible-exposed-infected-recovered (SEIR) framework. This model assumes no births or deaths. The subscript i denotes the index of the age strata. The parameter r_i denotes the rate of symptomatic infection for age class G_i . The parameter u_i denotes the relative susceptibility of age class G_i . We set values for r_i and u_i according to the means of the consensus estimates from Davies et al.

$$r=\{0.40, 0.25, 0.37, 0.42, 0.51, 0.59, 0.2, 0.76, 0.76\}$$

$$u=\{0.33, 0.37, 0.69, 0.81, 0.74, 0.8, 0.89, 0.77, 0.77\}$$

The infected class is decomposed into asymptomatic (I_A), symptomatic, pre-clinical (I_P), and symptomatic, clinical (I_C) classes order to reflect relevant aspects of SARS-Cov-2 epidemiology, namely that not all infected individuals show symptoms, and individuals are frequently quarantined upon presenting symptoms. We also decomposed the recovered class into separate compartments for those recovered from symptomatic infection, R_S , and those recovered from asymptomatic infection, R_A , in order to simplify calculations of total symptomatic and asymptomatic cases. This model framework allows us to impose assumptions about the infectivity of asymptomatic individuals and fully symptomatic individuals (b_A and b_C respectively) relative to the infected class likely responsible for the bulk of transmission (I_P).

$$\frac{dS_i}{dt} = -S_i u_i \beta \sum_{j=1}^9 C_{i,j} \frac{I_{Pj} + b_C I_{Cj} + b_A I_{Aj}}{N_j}$$

$$\frac{dE_i}{dt} = S_i u_i \beta \sum_{j=1}^9 C_{i,j} \frac{I_{Pj} + b_C I_{Cj} + b_A I_{Aj}}{N_j} - \delta_E E_i$$

$$\frac{dI_{P_i}}{dt} = r_i \delta_E E_i - \delta_P I_{P_i}$$

$$\frac{dI_{C_i}}{dt} = \delta_P I_{P_i} - \delta_C I_{C_i}$$

$$\frac{dI_{A_i}}{dt} = (1 - r_i) \delta_E E_i - \delta_A I_{A_i}$$

$$\frac{dR_S}{dt} = \delta_C I_{C_i}$$

$$\frac{dR_A}{dt} = \delta_A I_{A_i}$$

Here, C is the contact matrix, whose entries C_{ij} correspond to the mean number of contacts between individuals in the i -th and j -th age classes of G , δ parameters determine the mean amount of time that individuals spend in each class, and β is the transmission parameter.

We used this model to simulate a wide range of plausible epidemiological scenarios. Specifically, we considered values for b_C in $\{0.1, 0.5, 1\}$, values for R_0 in $\{2, 4, 6\}$, and values for the degree of homogeneous mixing, in $\{0, 0.5, 1\}$. In the sections below, we describe how we use to construct the contact matrix C . We set the values of the following model parameters according to published estimates [3]: $b_A = 0.5$, $\delta_E = \frac{1}{3}$, $\delta_P = \frac{1}{2.1}$, $\delta_C = \frac{1}{2.9}$, $\delta_A = \frac{1}{5}$. After constructing C and fixing these variables, we used numerical methods combined with the next-generation matrix approach [24] to calculate the value for β that corresponds to the value R_0 we wished to assume for each scenario.

Rescaling contact matrix

We used the ‘*socialmixr*’ [25] R package to retrieve the UK contact matrix from the POLYMOD study [16] with contacts binned according to the following age categories: $\{0-9, 10-19, \dots, 60-69, 70+\}$. We term this matrix A . No finer resolution was available for contacts involving individuals over the age of 70. However, in order to account for differences between individuals in the 70-79 and 80+ age classes in terms of relevant COVID-19 parameters, we synthesized a new matrix, B , that includes contacts for individuals in the 70-79 and 80+ age classes:

$$B_{i,70-79} = A_{i,70+} \frac{N_{70-79}}{N_{70+}}$$

$$B_{i,80+} = A_{i,70+} \frac{N_{80+}}{N_{70+}}$$

$$B_{70-79,j} = A_{70+,j}$$

$$B_{80+,j} = A_{70+,j}$$

Here N_x is the number of individuals in the entire U.S. in age class x .

Next, we constructed the contact matrix used in our model C by rescaling B to reflect our assumptions about mixing patterns.

$$C_{i,j} = \frac{(1 - \theta) \sum_{j=1}^9 B_{i,j}}{9} + \theta B_{i,j}$$

The quantity θ represents the degree of homogeneous mixing. When $\theta = 1$, contact patterns are identical to the POLYMOD contact patterns. When $\theta = 0$, contact rates are homogenous across age classes. Values of θ between 0 and 1 correspond to mixing patterns intermediate between the POLYMOD and homogenous scenarios. This rescaling procedure preserves the total number of contacts that each age class experiences while changing the identity of those contacts.

Model simulation

For each scenario in each county, we used the following conditions to initiate the model:

$$S_i = N_i - 4$$

$$E_i = 1$$

$$I_{P_i} = 1$$

$$I_{C_i} = 1$$

$$I_{A_i} = 1$$

$$R_{S_i} = 0$$

$$R_{A_i} = 0$$

The number of individuals within each age class for the county of interest is N_i .

We then simulated the model in R, using the ‘ode’ function in the ‘*deSolve*’ package[26] with the ‘*lsoda*’ integrator and a step size of 0.25. We truncated the simulation when

$$\sum_{i=1}^9 \frac{I_{P_i} + I_{C_i} + I_{A_i} + R_{S_i} + R_{A_i}}{S_i + E_i + I_{P_i} + I_{C_i} + I_{A_i} + R_{S_i} + R_{A_i}} = 0.2$$

and then extracted the number of individuals in each age stratified compartment.

Case estimation

We calculated the total number of symptomatic infections in each age class by the time the cumulative infection rate reaches 20% as $I_{P_i} + I_{C_i} + R_{S_i}$ at the end of the simulation. We then calculated the number of hospitalizations in each age class by multiplying the number of symptomatic infections in each age class by age stratified estimates [11] of hospitalization rates for symptomatic cases: {0.001, 0.003, 0.012, 0.032, 0.049, 0.102, 0.166, 0.243, 0.273}

We then calculated the number of ICU admissions in each age class by multiplying the number of hospitalizations by age stratified estimates[11] of the rate of ICU admission given hospitalization: {0.05, 0.05, 0.05, 0.063, 0.122, 0.274, 0.432, 0.709}.

Case distribution

We distributed cases originating in a given county to the healthcare systems of that county and other counties using the following algorithm.

- Let the county of origin be denoted as c_0 and the potential destination counties as

$$c_0, \dots, c_N$$

- Let the distances between the center of population of the county of c_0 and each potential destination county c_i be $d_{0,i}$

- We obtained the latitude and longitude of the center of population for each county from publicly available data from the 2010 U.S. census, and calculated pairwise distances between counties using R package ‘*geosphere*’ [27].
- We next removed all destination counties with $d_{0,i} > 400\text{km}$.
- We calculated a distance weight, y_i , for each remaining potential destination county as
$$y_i = \frac{1}{20} e^{\frac{d_{0,i}}{20}}$$
- We calculated a bed weight, z_i , for each county as the number of total hospital beds in c_i . For projections involving ICU admissions, we used the number of ICU beds instead of the number of hospital beds.
- We then calculated a composite weight, w_i , for each county as $w_i = \frac{y_i}{\sum_{j=0}^9 y_j} \frac{z_i}{\sum_{j=0}^9 z_j}$
- Lastly, cases originating in c_0 were then distributed to counties $c_0, \dots, c_N$
- proportion to $\frac{w_0}{\sum_{i=0}^9 w_i}, \dots, \frac{w_N}{\sum_{i=0}^9 w_i}$

‘Alternate optimistic’ and ‘alternate pessimistic’ scenarios

For the two scenarios in which we varied R_0 between counties according to the percentage of the population residing in rural areas, the value of R_0 for each county was calculated as:

$$R_0 = 2 + (R_{0_{max}} - 2) * \% \text{ population residing in urban area}$$

Instead of truncating our simulations at a 20% cumulative infection rate, we truncated our simulations when the following condition was met, indicating that the cumulative infections rate was equal to 20% of the herd immunity threshold:

$$\sum_{i=1}^9 \frac{I_{P_i} + I_{C_i} + I_{A_i} + R_{S_i} + R_{A_i}}{S_i + E_i + I_{P_i} + I_{C_i} + I_{A_i} + R_{S_i} + R_{A_i}} = 0.2 * (1 - \frac{1}{R_0})$$

Code availability

The code used to conduct analyses and generate figures is available at

<https://github.com/ianfmiller/covid19-burden-mapping>.

References

1. World Health Organization. 2020 Coronavirus disease 2019 (COVID-19) Situation Report 95.
2. CDC. 2020 COVIDView Week 13.
3. Davies NG *et al.* In press. Age-dependent effects in the transmission and control of COVID-19 epidemics. , 1–31.
4. Wang C, Horby PW, Hayden FG, Gao GF. 2020 A novel coronavirus outbreak of global health concern. *Lancet* **395**, 470–473. (doi:10.1016/S0140-6736(20)30185-9)
5. Holshue ML *et al.* 2020 First case of 2019 novel coronavirus in the United States. *N. Engl. J. Med.* **382**, 929–936. (doi:10.1056/NEJMoa2001191)
6. Liu Y, Gayle AA, Wilder-Smith A, Rocklöv J. 2020 The reproductive number of COVID-19 is higher compared to SARS coronavirus. *J. Travel Med.* , 1–4. (doi:10.1093/jtm/taaa021)
7. Wu JT, Leung K, Bushman M, Kishore N, Niehus R, Salazar PM De, Cowling BJ, Lipsitch M, Leung GM. 2020 Estimating clinical severity of COVID-19 from the transmission dynamics in Wuhan , China. *Nat. Med.* (doi:10.1038/s41591-020-0822-7)
8. Emanuel EJ *et al.* 2020 Fair Allocation of Scarce Medical Resources in the Time of Covid-19. *N. Engl. J. Med.* (doi:10.1056/NEJMs2005114)
9. White DB, Lo B. 2020 A Framework for Rationing Ventilators and Critical Care Beds

- During the COVID-19 Pandemic. *JAMA* (doi:10.1001/jama.2020.5046)
10. Hay JA, Haw DJ, Hanage WP, Metcalf CJE, Mina MJ. 2020 Implications of the Age Profile of the Novel Coronavirus.
 11. Ferguson NM *et al.* 2020 Impact of non-pharmaceutical interventions (NPIs) to reduce COVID- 19 mortality and healthcare demand.
 12. Gamio L, Cai W, Hassan A. 2020 Where the U.S. Stands Now on Coronavirus Testing. *New York Times*, 28 March. See <https://www.nytimes.com/interactive/2020/03/26/us/coronavirus-testing-states.html?action=click&module=Top Stories&pgtype=Homepage>.
 13. In press. The COVID Tracking Project. See <https://covidtracking.com/data>.
 14. Adolph C, Amano K, Bang-jensen B, Fullman N, Wilkerson J. 2020 Pandemic Politics : Timing State-Level Social Distancing Responses to COVID-19 *. *medRxiv*
 15. In press. covid-19-data. *New York Times*. See <https://github.com/nytimes/covid-19-data>.
 16. Mossong J *et al.* 2008 Social contacts and mixing patterns relevant to the spread of infectious diseases. *PLoS Med.* **5**, 0381–0391. (doi:10.1371/journal.pmed.0050074)
 17. Tedijanto C, Lipsitch M. 2020 Social distancing strategies for curbing the COVID-19 epidemic.
 18. U.S. Census Bureau. In press. 2018 American Community Survey 1-Year Estimates, Table DP05.
 19. U.S. Census Bureau. In press. 2010 U.S. Census, Table P2.
 20. American Hospital Association. In press. 2018 AHA Annual Survey Database.
 21. Lurie N, Saville M, Hatchett R, Halton J. 2020 Developing Covid-19 Vaccines at Pandemic Speed. *N. Engl. J. Med.* , NEJMp2005630. (doi:10.1056/NEJMp2005630)

22. Yang J *et al.* 2020 Prevalence of comorbidities in the novel Wuhan coronavirus (COVID-19) infection: a systematic review and meta-analysis. *Int. J. Infect. Dis.* (doi:10.1016/j.ijid.2020.03.017)
23. Deng X *et al.* 2020 Case fatality risk of novel coronavirus diseases 2019 in China. *medRxiv* (doi:10.1101/2020.03.04.20031005)
24. Diekmann O, Heesterbeek JAP, Metz JAJ. 1990 On the definition and the computation of the basic reproduction ratio R_0 in models for infectious diseases in heterogeneous populations. *J. Math. Biol. Math. Biol.* **28**, 365–382.
25. Funk S. 2020 socialmixr: Social mixing matrices for infectious disease modeling.
26. Soetaert K, Petzoldt T, Setzer RW. 2010 Package deSolve : Solving Initial Value Differential Equations in R. *J. Stat. Softw.* **33**, 1–25. (doi:10.18637/jss.v033.i09)
27. Hijmans RJ. 2019 goesphere: Spherical Trigonometry.

Chapter III: Assessing the risk of vaccine-driven virulence evolution in SARS-CoV-2

Foreword

This chapter was published as a research article in Royal Society Open Science in January of 2022:

Miller, I.F. and Metcalf C. J. E. 2022. Assessing the risk of vaccine-driven virulence evolution in SARS-CoV-2. *R. Soc. Open Sci.* 9211021211021 <http://doi.org/10.1098/rsos.211021>

Abstract

The evolution of SARS-CoV-2 virulence, or lethality, threatens to exacerbate the burden of COVID-19 on society. How might COVID-19 vaccines alter selection for increased SARS-CoV-2 virulence? Framing current evidence surrounding SARS-CoV-2 biology and COVID-19 vaccines in the context of evolutionary theory indicates that prospects for virulence evolution remain uncertain. However, differential effects of vaccinal immunity on transmission and disease severity between respiratory compartments could select for increased virulence. To bound expectations for this outcome, we analyze an evo-epidemiological model. Synthesizing model predictions with vaccine efficacy data, we conclude that while vaccine driven virulence remains a theoretical possibility, the risk is low if vaccines provide sustained robust protection against infection. Furthermore, we found that any increases in transmission concomitant with increases in virulence would be unlikely to threaten prospects for herd immunity in a highly immunized population. Given that virulence evolution would nevertheless impact unvaccinated individuals and populations with low vaccination rates, it is important to achieve high vaccination rates worldwide and ensure that vaccinal immunity provides robust protection against both infection and disease, potentially through the use of booster doses.

Introduction

Since its emergence in late 2019, the SARS-CoV-2 virus has spread globally. As of October 2021, it has resulted in over 243 million cases of its associated disease, COVID-19, and over 4.9 million deaths [1]. Beyond the morbidity and mortality associated with the pandemic, nations and sub-national states heavily affected by COVID-19 have experienced catastrophic economic collapse [2], critical pauses in educational services, and pervasive psychological damage. Generating herd immunity through vaccination has been consistently identified as the only acceptable course of action for mitigating the pandemic and allowing society to begin the recovery process. The race for SARS-CoV-2 vaccines has proceeded at an unprecedented pace. Following promising results from phase 3 trials [3–6] several vaccines have been authorized for use. While clinical trials provide the evidence necessary to confirm the ability of vaccines to safely prevent disease in individuals, potential population level and longer-term consequences of vaccine introduction, particularly viral evolution, should also be considered. Potential negative outcomes include vaccine escape via antigenic evolution, which would result in a decrease or loss of vaccine efficacy [7], and the evolution of increased virulence, which could result in more severe health outcomes and a higher infection fatality ratio amongst unvaccinated individuals. The recent emergence of the *alpha* and *delta* variants has drawn attention to the threat posed by SARS-CoV-2 virulence evolution. Some evidence indicates that the *delta* variant may be linked to increased infection fatality rates [8], although there is still considerable uncertainty [9,10].

In this paper, we assess the potential for vaccines to drive the evolution of SARS-CoV-2 virulence. First, we review the theory surrounding the evolution of virulence evolution as it relates to SARS-CoV-2. Next, we consider the effects of COVID-19 vaccines in this theoretical framework, evaluating current evidence and assessing the potential for selection for increased

virulence using an evo-epidemiological model. Finally, we synthesize our findings and generate recommendations for minimizing this risk, a question of growing importance in the happy circumstance of ever greater deployment of vaccines globally.

The theory of virulence evolution

The evolution of pathogen virulence, which for the purposes of this paper we define as the rate of disease associated mortality, has intrigued scientists for decades. In the absence of any associated costs, pathogens are expected to evolutionarily maximize both the rate at which they transmit from infected hosts, and the duration of time during which a host is infectious. Because mortality (and in some cases severe symptoms) curtails opportunities for transmission, virulence alone is never adaptive for pathogens, and is at best selectively neutral (e.g. when host mortality occurs after the host infectious period). However, for many pathogens, host damage either can enhance transmission (e.g. by inducing coughing) or is an unavoidable consequence of or requirement for transmission (e.g. cellular damage resulting from viral replication). Thus, selection for increased virulence can occur as a result of selection for increased transmission [11–13], and indeed, only as a result of selection for increased transmission.

From this evolutionary theory, it is evident that two conditions must be met for virulence evolution to occur. First, increased transmission must be biologically feasible, and must be determined by the genetics of the pathogen (e.g., it must be heritable). Second, evolutionary increases in transmission must lead to increases in virulence (e.g. virulence must be an unavoidable consequence of transmission). These two conditions determine whether virulence evolution is possible, but they are not informative about the limits to virulence evolution, i.e., how destructive the pathogen has the potential to become, in terms of host mortality.

There are two major sets of factors that limit virulence evolution. The first involves limits to transmission evolution. Because virulence can only be selected for in association with transmission, any biologic, metabolic, or other limits to transmission evolution (such as limits to the rate of protein synthesis or viral assembly) necessarily constrain virulence evolution [14,15]. The second set of factors limiting virulence evolution encompasses the effects of host mortality on transmission rate. If neither symptoms nor disease-associated death shorten the duration of transmission, then virulence does not reduce pathogen fitness and its evolution is not constrained [16; Fig 1, yellow lines]. Conversely, if symptoms or disease can truncate the infectious period, then the potential for a trade-off between virulence and transmission that constrains the evolution of these two traits emerges.

To put this formally, for many pathogens, incremental increases in transmission are hypothesized to bear increasing costs of damage, leading to a positive, saturating relationship between mortality (or severity) rate and the maximum attainable transmission rate. This relationship -- the canonical 'virulence transmission tradeoff' -- has the effect of limiting the evolution of virulence (Fig. 1, purple lines). The saturating nature of this trade-off curve makes an intermediate degree of virulence evolutionarily optimal, as the benefits of increased transmission are balanced against the costs of host death (or other severe outcomes truncating transmission). If the trade-off curve does not saturate (Fig. 1, teal lines), then any decreases in transmission time due to increased virulence are more than compensated for by increases in transmission rate, and the evolution of virulence is not constrained. It is important to note that the trade-off curve bounds the set of possible virulence and transmission strategies. Evolutionary changes can occur on the interior of this set with unconstrained increases and/or decreases in both virulence and transmission, and as such the presence or shape of a trade-off may not be

apparent until transmission is maximized for a given virulence strategy and evolution begins to ‘trace the curve.’ Direct evidence for the existence of virulence-transmission trade-offs is limited, but suggestive evidence has been found in many systems [13].

These theoretical frameworks can be used to assess whether the evolution of increased SARS-CoV-2 virulence is possible and to identify factors limiting the extent to which this could occur.

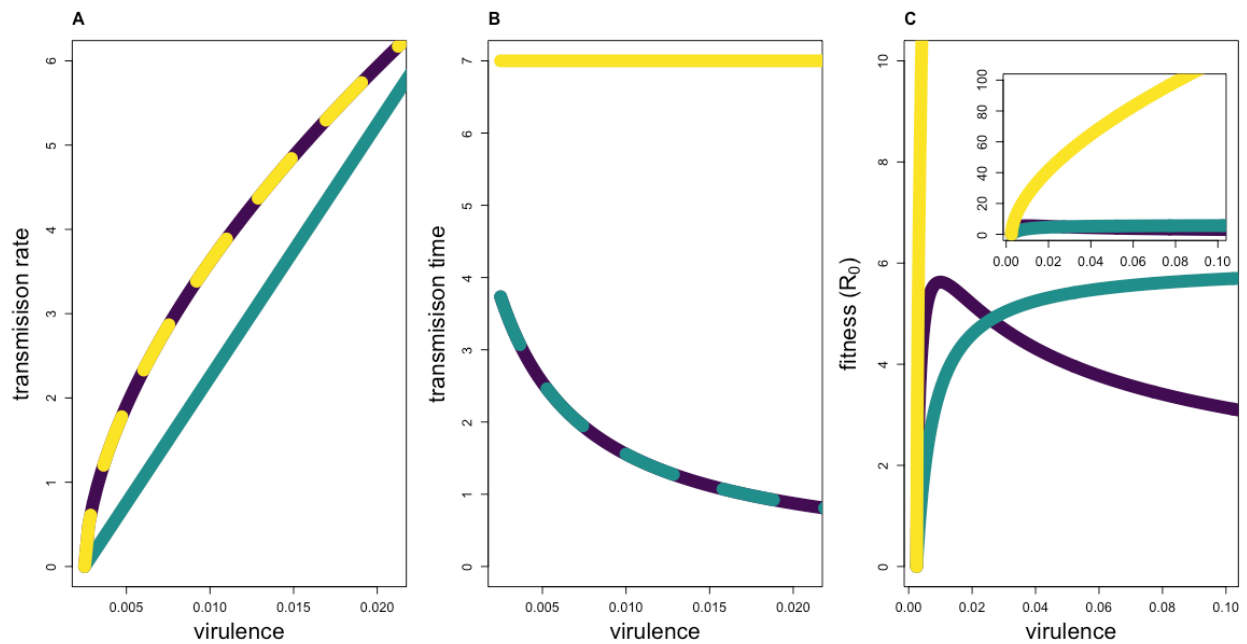


Figure 1: Virulence-transmission trade-offs. (A) Different possible shapes for the relationship between transmission and virulence. The curves define the maximum transmission rate attainable for a given virulence strategy (interpretable as e.g., the infection fatality ratio). The region under each curve represents the set of all possible combinations of transmission rate and virulence for that trade-off shape. (B) The relationship between virulence and transmission time. (C) The relationship between virulence and fitness (in a completely susceptible population, equivalent to R_0). Purple lines show relationships consistent with a virulence-transmission trade-off. Teal lines illustrate a non-saturating relationship between virulence and transmission. Yellow lines correspond to the scenario in which virulence does not decrease transmission time (for example, if mortality occurs after the infectious period has ended).

Evaluating potential for virulence evolution in SARS-CoV-2

The evidence surrounding differences in transmission and virulence between the ancestral SARS-CoV-2 strain and the emergent *alpha* (i.e. *B.1.1.7*) and *delta* (*B.1.617.2*) variants sheds light on whether the two conditions necessary for SARS-CoV-2 virulence evolution are met. These variants represent separate branches of viral evolution stemming from the ancestral strain [17,18]. The *alpha* variant was designated as a variant of concern by the World Health Organization in December 2020, and the *delta* variant received the same designation in May 2021 [19].

Current evidence surrounding these two variants gives a clear indication that the first condition (the feasibility and heritability of increased transmission rate) is satisfied. The *alpha* variant has been identified as being 43-82% more transmissible than ancestral genotypes [20]. An even more dramatic increase in transmission has been observed in *delta*, which is estimated to be at least twice as transmissible as *alpha* [21,22], and has become the predominant variant worldwide [1]. Other variants and mutations have also been linked to increased transmission. For example, the *D614G* mutation is hypothesized to be associated with increased transmission due to the rapid increase in its frequency [23,24], enhanced replication in vitro [24,25] and increased viral titers in vivo [26].

The second condition (virulence being a consequence of increased transmission) necessary for virulence evolution is similarly supported by patterns of increased mortality associated with the *alpha* and *delta* variants. The *alpha* variant is associated with an approximately 55% increase in infection fatality rate over the ancestral strain [27], and the *delta*

variant has been linked to even greater increases in virulence over *alpha*. A retrospective cohort study examining records from Ontario, Canada, found that infection with *delta* strain was associated with a 133% increase in the risk of death compared to infection with the ancestral strain [8]. Likewise, an observed increase in the risk of hospitalization in patients infected with the *delta* vs. *alpha* strain [28] further points to an incremental increase in virulence in *delta*. However, several studies cast doubt on claims that *delta* is more virulent than *alpha*. In the United States, the proportion of children hospitalized with COVID-19 that went on to experience severe outcomes such as intensive care unit (ICU) admission or death were not significantly different before and after the emergence of *delta*, although non-statistically significant increases (perhaps due to very small sample sizes) in the proportion of patients who died or required invasive mechanical ventilation were noted [9]. Likewise, the proportion of adults hospitalized with COVID-19 who died or were admitted to an ICU did not significantly change in the wake of *delta*'s emergence as the dominant strain, but the proportion of adults hospitalized with COVID who were aged 18-49 years rose, and the proportion of adults 50 and over hospitalized with COVID who experienced ICU admission or death trended upward (but was not significantly greater) [10]. Furthermore, beyond evidence surrounding *alpha* and *delta*, it is worth noting that not all increases in transmission have been tied to increases in virulence. In the case of the *D614G* mutation, clinical data does not indicate any change in the severity of disease [29]. See Alizon et al. [30] and Otto et al. [31] for a more detailed discussion of variants and how their traits relate to virulence evolution.

Clear evidence of past evolutionary increases in SARS-CoV-2 virulence suggest that future increases may be possible. Given this possibility, could either biological limits to transmission or a virulence-transmission trade-off constrain how virulent SARS-CoV-2 could

become? To date, no limits to the evolution of SARS-CoV-2 transmission rate have been described. However, despite extremely large viral population sizes, only a few evolutionary increases in transmission have been detected, although this pattern should not necessarily be viewed as indicative of future trends, given the many surprises SARS-CoV-2 evolution has produced to date. Turning to the potential for a virulence-transmission trade-off to limit the evolution of virulence, several threads of epidemiological and clinical evidence indicate that disease associated mortality does not significantly decrease transmission time in SARS-CoV-2 infection [30]. The infectious period for COVID-19 infection is known to start before symptom onset [32], the majority of infected individuals experience only mild symptoms [33], and death usually occurs several weeks after initial infection [34]. These patterns don't preclude the existence of a virulence-transmission trade-off, but they do suggest that if such a trade-off does exist, virulence likely limits transmission time through a mechanism other than death. This could occur if increased virulence is associated with increased symptom frequency (i.e. a lower asymptomatic rate, shown not to be the case for *alpha* [35]), more severe initial symptoms for those who are symptomatic, or faster symptom onset. Due to widespread public knowledge of SARS-CoV-2 circulation and non-pharmaceutical interventions such as case isolation (which has been shown to decrease transmission time as evidenced by a shortening of the serial interval [36]), individuals infected with a more virulent variant would presumably more frequently and/or more rapidly modify their behavior to reduce onwards transmission than individuals infected with a less virulent strain. Overall, while a virulence-transmission trade-off could emerge from the relationship between transmission rate and mortality rate, or these potential links between severity, symptoms, and transmission time, current data are insufficient to conclusively determine the existence of such a trade-off, its shape, or where current the SARS-CoV-2

virulence and transmission rates fall on the curve or within the set of strategies that it bounds. It is also important to note that many factors other than a virulence transmission trade-off may influence the evolution of epidemiologically relevant SARS-CoV-2 traits, including virulence [30,31,37].

COVID-19 vaccines and virulence evolution

Current evidence indicates that the evolution of increased SARS-CoV-2 virulence is possible, as transmission and virulence may be linked, but the constraints determining the extent to which virulence might increase are not well resolved. Considering this prospect is nevertheless important, because vaccinal immunity can drive selection for increased virulence in certain circumstances. If the evolution of transmission is unconstrained, then selection always favors increased transmission (and thus increased virulence if the two are associated) regardless of the effects of immunity, which can only modify the strength of selection. However, if virulence evolution is constrained by a virulence-transmission trade-off, vaccines might have the potential to determine the strength and directionality of selection for increased virulence by removing or reducing the costs of virulence to the pathogen. In this case, theory predicts that immunity which reduces disease but not transmission [38], or reduces disease to a greater extent than transmission [39], can drive the evolution of increased virulence. This points to a potential risk for certain vaccines to drive the evolution of more virulent pathogens. While there is evidence for some aspects of vaccine driven pathogen evolution in several human diseases, such as the emergence of vaccine-resistant lineages of *Streptococcus pneumoniae* [40] and *Bordetella pertussis* [41], to date there have been no increases in virulence definitively linked to vaccine use in humans [42]. However, several pieces of evidence highlight the existence of this risk: Evolutionary increases

in virulence driven by vaccination have been observed in a mouse model of malaria [43] and in an oncogenic herpes virus infecting commercially raised chickens [44]. It is important to note that some factors present in these two examples of vaccine-driven virulence evolution are absent in the case of SARS-CoV-2. For instance, in the latter example, viral virulence was extremely high (60-100% infection fatality rate) prior to vaccine-driven evolution, and vaccinal protection against infection was extremely poor.

Vaccinal immunity to SARS-CoV-2 could open the door for virulence evolution if vaccination reduces disease to a greater extent than transmission. The contrasting consequences of SARS-CoV-2 infection in the upper respiratory tract (URT) and lower respiratory tract (LRT) provide one potential mechanism whereby the effects of immunity on these factors could be separated, and we use this to frame discussion of how such separation might drive virulence evolution. Evidence to date indicates that protection in the LRT could reduce disease severity, potentially reducing the costs of increased transmission. Protection in the URT, the primary location of infection colonization, might lead to a reduction in infection risk, potentially even to the level of sterilizing immunity, negating any gains in transmission rate the virus might be able to acquire via increased virulence [45]. The partitioning of virulence and transmission effects between respiratory tract compartments is known to exist for other respiratory diseases such as influenza [46,47]. In SARS-CoV-2, the mechanisms underlying the separation of virulence and transmission effects between the LRT and URT are increasingly resolved. The pattern of higher viral infectivity in the URT compared to the LRT reflects a decreasing gradient of angiotensin-converting enzyme 2 (ACE2, the receptor protein utilized by SARS-CoV-2 for cellular entry) expression from the URT to the LRT [48].

Given this structure, if vaccines have differential effects in the LRT and URT, then they may have differential effects on transmission and virulence, opening the way for them to drive virulence evolution. Preliminary evidence suggests that protective effects of SARS-CoV-2 vaccines might indeed differ between respiratory compartments. Non-human primate challenge studies investigating the efficacy of early COVID-19 vaccine candidates found that vaccinal immunity reduced viral replication in the LRT to a greater extent than in the URT [45,49]. The patterns of lower URT than LRT protection for these candidate vaccines may be rooted in the type of immune responses they induce. All are delivered intramuscularly, which generally and predominantly stimulates the production of IgG antibodies. The LRT system is primarily protected by these IgG antibodies, while the URT is primarily protected by IgA antibodies involved in mucosal immunity [45]. Other tissues where SARS-CoV-2 infection can cause acute injury [50] are protected by IgG antibodies, indicating immunological protection against damage in the lower respiratory and the rest of the body might be correlated.

While the scale of vaccine distribution indicates that vaccines will be a critical factor shaping the landscape of immunity that drives SARS-CoV-2 evolution in many parts of the world, naturally acquired immunity could also contribute to selective pressures. Evidence suggests that similarly to SARS-CoV-2 vaccines, natural immunity provides greater LRT protection than URT protection, although the differential might not be as large. In macaques re-challenged with SARS-CoV-2, immunity eliminated or significantly reduced viral replication in the LRT, and reduced replication in the URT, but to a lesser extent [51]. Consistent with this pattern, a longitudinal study of unvaccinated healthcare workers in the UK found that 0/1246 individuals with anti-spike protein IgG antibodies (from previous infection) and 89/11052 seronegative individuals became symptomatically infected, indicating that natural immunity

provides robust protection against disease [52]. The same study also found that natural immunity provides substantial but incomplete immunity against infection, as the incidence of asymptomatic infection was observed to be roughly four times higher in the seronegative group compared to the seropositive group [52]. Natural infection stimulates the production of both IgG and IgA antibodies [45], which suggests that natural infection could generate more balanced URT and LRT protection than vaccination, making the evolution of increased virulence a less likely outcome of selection imposed by natural immunity alone.

Bounding the effects of COVID-19 vaccines on selection for increased virulence

The unprecedented scale and speed at which SARS-CoV-2 vaccines are being deployed point to considerable value in bounding expectations on selection for increased viral virulence under different vaccine characteristics. The uncertainty surrounding the plausibility of and limits to SARS-CoV-2 virulence evolution complicates predictions, but it is possible to identify conditions associated with a pessimistic scenario in which vaccination has the most potential to drive selection for increased virulence. These conditions include the heritability of and thus evolutionary potential for increased transmission, a positive association between transmission and virulence, no biological limits to transmission, diminishing gains in transmission rate with increasing virulence, and increases in the removal rate of infectious individuals proportional to increases in virulence. These last two conditions constitute a virulence-transmission trade-off, and while they intentionally do not represent a specific mechanism, they are broadly consistent with a variety of ways in which a trade-off might emerge (e.g. faster symptom onset, more

frequent isolation of symptomatic individuals). A scenario in which the relationship between transmission in virulence is positive but non-saturating would be even more pessimistic, but in this case selection for increased virulence would occur regardless of the effects of vaccination.

We develop a general theoretical framework to explore the degree to which different combinations of vaccine coverage and vaccinal protection in the URT and LRT (represented by parameters $r_{U,V}$ and $r_{L,V}$) might shape the evolution of virulence under the conditions associated with this ‘pessimistic’ scenario (Fig. 2). We take an adaptive dynamics approach to identify how vaccine characteristics map to different values of evolutionarily stable virulence (i.e. pathogen virulence at evolutionary equilibrium) and various evo-epidemiological outcomes including unbounded selection for increased virulence, evolution-proof herd immunity, and non-evolution-proof herd immunity. To accommodate our mechanism-free assumption of a virulence-transmission trade-off, we assume susceptible-infectious-quarantined-convalescent dynamics. The rate at which infected individuals become quarantined is proportional to virulence, reflecting the assumption that increased virulence would lead to faster symptom onset and/or increased symptom severity. While our model formulation does not explicitly incorporate all details of COVID-19 epidemiology (e.g. pre-symptomatic transmission), its general form broadly captures all relevant dynamics. To explore how the strength and direction of selection for increased virulence changes with assumptions about where the virulence of *delta* falls on the virulence-transmission trade-off curve, we replicate our analyses across a range of assumptions about how optimal virulence, α_{optim} , compares to the levels of virulence associated with *alpha* (α_{alpha}) and *delta* (α_{delta}), which is the most-fit variant detected to date. Specifically, we investigate scenarios in which α_{optim} is intermediate between α_{delta} and α_{alpha} , equal to α_{delta} , or greater than α_{delta} . While *delta* has yet to be definitively linked to an increase in virulence, we assume

$\alpha_{\alpha} < \alpha_{\delta}$, in line with our aim to investigate a pessimistic scenario. our analysis is motivated by SARS-CoV-2, but separation of contributions to virulence and transmission from different compartments (e.g., in URT and LRT) is likely relevant for other viruses.

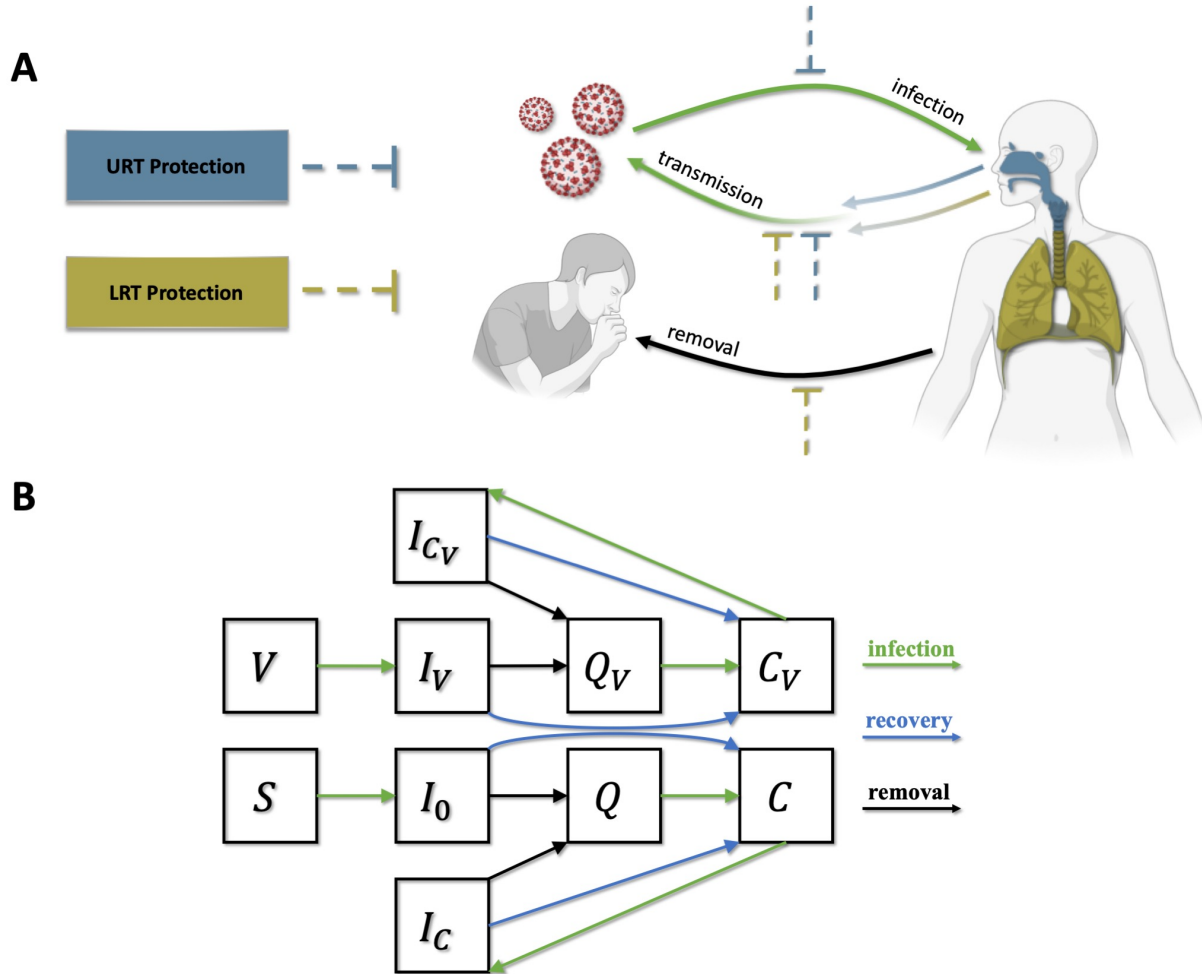


Figure 2: Model Schematic. (A) The effects of immunological protection (either vaccinal or naturally acquired) in the URT and LRT on transmission, infection, and disease severity. Immunological protection in the URT and/or LRT decreases the rate of onward transmission. Additionally, URT protection decreases the rate of infection, and LRT protection decreases virulence, which we assume to be proportional to the rate of removal from the transmission pool. (B) Structure of the epidemiological model. Susceptible (S) and vaccinated individuals (V) become infected (to classes I_0 and I_V respectively) in a density dependent manner. Infected individuals can be removed to a quarantined class (Q or Q_V). We assume that quarantine occurs

before death in fatal infections, and as such ignore disease associated mortality. Infected and quarantined individuals recover to a convalescent class with either naturally acquired immunity (C) or a combination of natural and vaccinal immunity (C_V). Convalescent individuals can become reinfected and return to an infected class (I_C or I_{C_V}). All infected classes contribute to transmission.

Our analyses reveal several important relationships between vaccinal immunity and the degree of evolutionarily stable virulence, α_{ESS} (Fig. 3). We observe that α_{ESS} is highly sensitive to vaccinal protection in the LRT and increases with rising values of $r_{L,V}$. This same pattern does not hold for upper respiratory tract protection, as α_{ESS} is minimally sensitive to the value of $r_{U,V}$. For any given combination of vaccine effects ($r_{U,V}, r_{L,V}$), α_{ESS} rises with the value of α_{optim} , and to a lesser extent also increases with vaccine coverage.

Beyond the determining the value of α_{ESS} , our analyses connect vaccinal immunity to qualitative evo-epidemiological outcomes, capturing the larger overall benefits of vaccines (Fig. 3). Our results map to four outcomes, listed here from worst to best: endemic SARS-COV-2 with unbounded selection for increased virulence (Fig. 3, grey regions), endemic SARS-COV-2 with an increase in virulence to α_{ESS} (Fig. 3, orange to purple regions), herd immunity (within the theoretical population in consideration) if epidemiological equilibrium is reached before an evolutionary increase in virulence to α_{ESS} occurs (Fig. 3, hashed regions), and evolution-proof herd immunity (Fig. 3, white regions). Across all assumptions about the optimum degree of virulence, the latter two outcomes can only be achieved when URT protection is strong and vaccine coverage is high, with the ideal outcome of evolution-proof herd immunity requiring higher levels of URT protection. These positives outcomes were achievable across all assumed values of α_{optim} for similar degrees of URT and LRT protection. We observed similar patterns

of virulence evolution and evo-epidemiological outcomes when assuming that LRT infection contributes minimally to transmission and for alternate assumptions about the strength of the protective effects of natural immunity in the URT and LRT (Figs. S3-5).

Clinical trials and real world vaccine efficacy data provide information about the effects of COVID-19 vaccines that we can compare to our model predictions to shed light on how we might expect virulence evolution to unfold. Evidence from early clinical trials suggested that COVID-19 vaccines provide robust protection against disease. Two mRNA vaccines, BNT162b2 and mRNA-1723, were shown to be 95% and 94% effective in preventing COVID-19 respectively while also exhibiting promise of preventing severe symptoms [3,4]. A recombinant adenovirus vaccine, ChAdOx1 had an efficacy of 62-90% in clinical trials carried out in Brazil, South Africa, and the United Kingdom [5]. Another recombinant adenovirus vaccine, Gam-COVID-Vac, or Sputnik V, exhibited an efficacy of 92% [6]. This strong protection against severe disease appears to hold against the *delta* variant as evidenced by real world data. In a large test-negative study in Canada, mRNA-1723, BnT162b2, and ChAdox1 were shown to be 98%, 97%, and 91% effective against the *delta* variant in preventing hospitalization and death after two doses [53].

Clinical trials provided more limited information about the infection limiting effects of vaccines, but the results were nonetheless promising. The ChAdOx1 vaccine was shown to reduce the incidence of PCR confirmed infection by 67% [54], consistent with a moderately strong infection blocking effect. An even more striking result was observed for the BNT162b2 vaccine, which has been shown to be 95.3% effective in preventing infections [55]. Real world data indicates that vaccines may be less effective against the *delta* variant in preventing transmission vs. severe disease. The test-negative study in Canada found that after two doses,

mRNA-1723, BNT162b2, and ChAdOx1 were 95%, 92%, and 87% effective against delta in preventing symptomatic infection respectively [53]. Data from England indicate slightly lower efficacy in protection against symptomatic infection, with two doses of BNT162b2 and ChAdOx1 estimated to be 88% and 67% respectively [56]. Concerningly, the efficacy of vaccination against infection appears to wane significantly over time [57,58]. Booster shots have been shown to be effective in restoring robust protection at least temporarily [59], and could provide a means for continued vaccinal protection against infection in the long term. Data on the extent to which SARS-COV-2 vaccines reduce onwards transmission is currently limited.

As a whole, this evidence suggests that COVID-19 vaccines confer a very high degree of LRT protection, and likely a significant amount of URT protection, although it is not possible at this time to map vaccines to a specific combination of protective effects $(r_{U,V}, r_{L,V})$, and the emergence of vaccine-resistant strains may decrease vaccine efficacy [60]. Given this combination of effects, our model suggests that 1) patterns of virulence evolution are likely to be highly sensitive to protective effects of vaccination and the optimum degree of virulence, and thus are impossible to predict at this time, 2) the optimal level of virulence, α_{ESS} , is expected to increase slightly with vaccine coverage, 3) existing vaccines likely significantly reduce transmission and could, if administered to a sufficient proportion of a population, generate herd immunity even under conditions where virulence evolution could occur (noting that waning vaccinal protection could jeopardize this outcome), and 4) these positive outcomes would remain attainable even if α_{optim} is somewhat higher than α_{delta} .

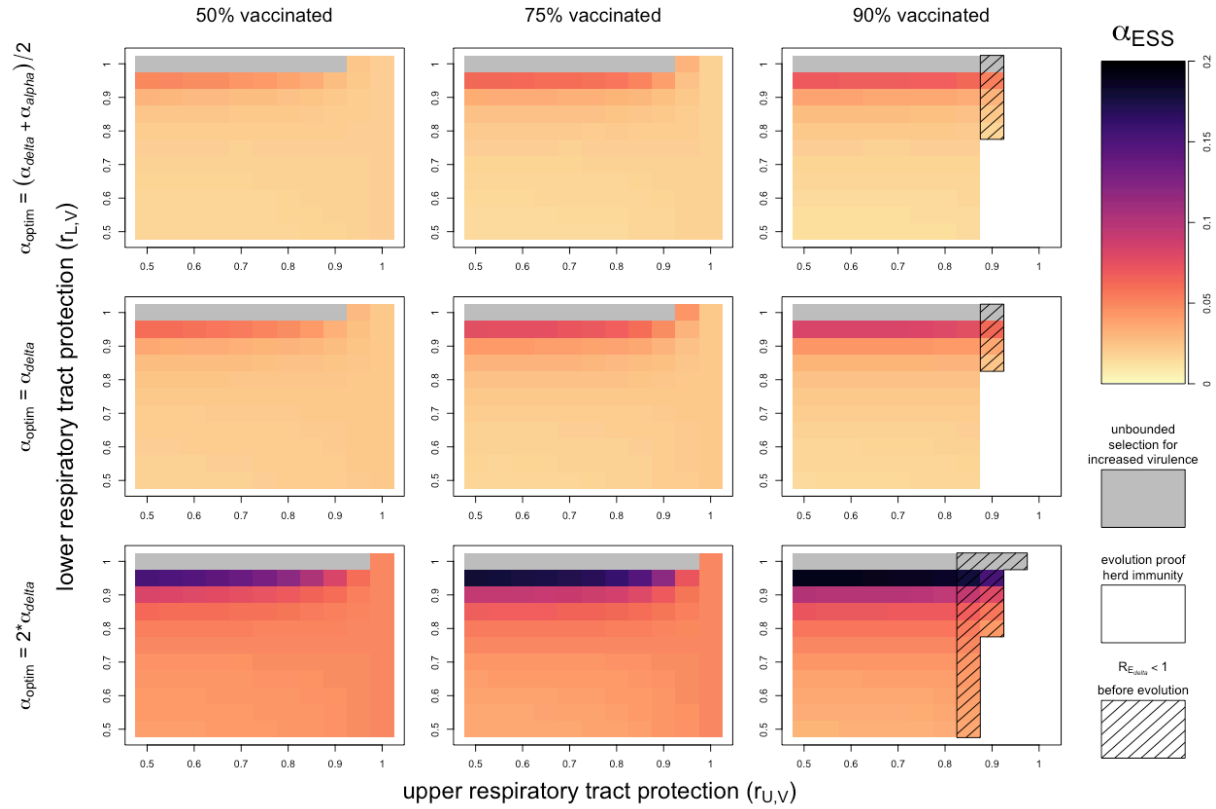


Figure 3: Virulence evolution and evo-epidemiological outcomes associated with vaccinal immunity. Panels show the evolutionarily stable virulence strategy resulting from different combinations of vaccinal protection in the LRT and URT. Each column corresponds to a different level of vaccine coverage, and each row corresponds to a different assumption about how the value of α_{optim} compares to α_{delta} and α_{alpha} . Darker colors indicate more virulent evolutionary stable strategies. Grey regions correspond to scenarios in which no evolutionary stable virulence strategy exists and increased virulence is selected for without bounds. White regions correspond to scenarios in which vaccinal (and natural) immunity are sufficient to achieve herd immunity (within the theoretical population in consideration) without the possibility of virulence evolution leading to the persistence of the pathogen. Hashed lines indicate that vaccinal and natural immunity may lead to herd immunity before virulence evolution takes place, but that increases in virulence are selected for and can erode herd immunity. In all panels natural immunity effects are set to $r_{U,C} = 0.5$, $r_{L,C} = 0.75$ and the lower and upper respiratory tracts contribute equally to transmission ($\varepsilon = 0.5$).

Discussion

As the global spread of SARS-CoV-2 continues and the widespread rollout of vaccines is underway, evaluating the risk of vaccine-driven virulence evolution increases in priority. We reviewed the theoretical drivers of virulence evolution, distilled a set of conditions necessary for the evolution of increased virulence, and evaluated them in the context of SARS-CoV-2, concluding that further evolution of SARS-CoV-2 virulence is plausible, but not certain. While the limits to SARS-CoV-2 evolution are not clear, several mechanisms could potentially result in vaccines driving selection for or against increased virulence. Using an evo-epidemiological model, we determined that under a pessimistic set of assumptions, existing vaccines could potentially select for increased virulence, but that if this were to occur, they would still generate herd immunity if administered to a large percentage of the population. This work builds upon the theoretical analyses of SARS-CoV-2 virulence evolution presented in many previous studies [30,37] by extending the same fundamental concepts to the issue of vaccine-driven evolution.

The concept of herd immunity in the context of our results warrants careful consideration. Herd immunity does not necessarily correspond to a “COVID-free” world [61]. Even if a population has immunological characteristics that render $R_E < 1$, spillover transmission from other populations could lead to small outbreaks of SARS-CoV-2, especially in sub-populations with low vaccination rates. However, herd immunity can prevent sustained within-population transmission. Another important detail deals with the temporal aspects of herd immunity. Although vaccines could create herd immunity in the short term, a build-up of susceptible individuals due to births and immunological waning might result in the loss of this population-level protection, leading to epidemics. While we do not explicitly incorporate waning or demographic processes into our predictions, interpreting the values of $r_{U,V}$ and $r_{L,V}$ as population

means that encompass decreases in efficacy over time allows for an approximate estimate of the impacts of waning (see methods).

The large degree of uncertainty surrounding the plausibility of vaccine driven virulence evolution, combined with our finding that vaccines could lead to herd immunity even if they select for increased virulence, suggests no cause for immediate concern about the risks posed by vaccines regarding virulence evolution. Additionally, the burden of increased virulence could be significantly decreased if virtually all primary infections occur in childhood, resulting in mild symptoms. In the absence of virulence evolution, this pattern is predicted to decrease the severity of SARS-CoV-2 to a level similar to other endemic human coronaviruses [62]. However, several actions should be taken to mitigate any risks. First, the protective effects of all SARS-CoV-2 vaccines in the URT and LRT, and how these effects map to reductions in infection and transmission, should be evaluated, further noting that this categorization may also be a simplification, and other mechanisms may be at play and should be investigated. The use of any vaccine that does not significantly reduce contagion should be carefully considered. Second, as more vaccines and more data become available, governments should prioritize the use of vaccines (including booster doses) that provide robust protection against both disease and transmission. Third, vaccination programs should aim to immunize a large percentage of the population as quickly as possible, as herd immunity, which could mitigate selection for increased virulence, is only possible at high vaccine coverage (in the absence of non-pharmaceutical interventions).

The benefits of transmission/infection reducing immunity stemming from vaccinal URT protection extend beyond minimizing selection for increased virulence and generating herd immunity. These effects, along with other measures, such as non-pharmaceutical interventions,

would also decrease effective viral population size, diminishing the impact of selection, the genetic diversity of the virus, and thus the likelihood of vaccine driven virulence evolution [37]. However, sources of genetic diversity could exacerbate risks of viral evolution. Significant viral diversity can be generated in chronically infected immunocompromised patients, and enhanced infection control procedures have been recommended to limit the spread of mutations from these individuals. Animal reservoirs are another potential source of genetic diversity [63]. SARS-CoV-2 epidemics in mink farms have already been linked to the generation of novel mutations [64] and animal to human transmission [65]. Other domestic and agricultural animal populations, especially those maintained at high density, should be actively monitored for SARS-CoV-2 transmission. Wild animal populations also have potential to harbor sustained viral transmission but present significant challenges for implementing control measures. Species that are known to be competent hosts for coronaviruses and regularly come into contact with humans should be prioritized for monitoring.

If vaccines were to drive the evolution of increased virulence, protection against disease in vaccinated individuals may or may not erode, but other groups would bear greater impacts [39]. Specifically, unvaccinated individuals would bear the consequences of increased virulence without any vaccinal protection to offset disease severity. Vaccine driven evolution could also amplify healthcare disparities between populations if a SARS-CoV-2 strain with increased virulence evolves in a population with a high vaccination rate, and spills over into a population with a low vaccination rate. Beyond seeking to reduce disparities in vaccination, countries with the highest vaccine coverage should continue to engage in virulence monitoring to detect emergent variants with increased virulence, and if such variants arise, enact containment measures to mitigate negative consequences for countries with limited access to vaccines.

Immunological waning is only implicitly incorporated in our analyses, but it is important to note that the resulting loss of vaccinal and/or natural immunity over time could increase both the effective viral population sizes and likelihood of SARS-CoV-2 becoming endemic [62,66,67], while also altering the selective forces acting on pathogen virulence. Future analyses should explore how waning immunity might alter selection for increased virulence as evidence regarding the longevity of immunity emerges. Booster vaccine doses offer a potential solution to mitigate waning vaccinal immunity. If waning vaccinal immunity results in a decrease in protection against infection but does not reduce protection against disease, then booster vaccine doses should be evaluated primarily on their ability to prevent infection and transmission.

While our analyses give approximate expectations under a specific set of assumptions, robust expectations about the likelihood, magnitude, and trajectory of virulence evolution remain elusive. We assumed a conventional saturating positive relationship between transmission and virulence. However, the shape, nature, and existence of any trade-offs governing virulence evolution in SARS-CoV-2 are currently unknown, and the form of true relationship could modify the direction and magnitude of selection acting on virulence. Assessments of the risk of virulence evolution should be updated as new findings emerge, both by directly monitoring the virulence of the virus, and by better characterizing the nature of the tradeoff. To monitor virulence, infection fatality ratios associated with circulating SARS-CoV-2 strains should continue to be surveilled to detect early evidence of any evolutionary trends. Stratifying these observations by age and immunological status (naïve, natural immunity, vaccinal immunity) would allow for changes in population-wide infection fatality rates to be properly attributed changes in virulence or other factors. Decreases in fatality rates tied to medical advances will complicate this monitoring, especially as they vary globally, but animal or cell-culture models might provide a

means for standardized measurement. Trade-offs affecting the evolution of SARS-CoV-2 transmission can be characterized through comparative epidemiological analyses and clinical studies of within-host viral dynamics.

While we focus on the evolution of SARS-CoV-2 virulence and transmission in this paper, other aspects of viral evolution may also affect COVID-19 severity. For example, antigenic evolution may could have implications for virulence. This could occur through either direct effects, such as antigenic changes altering the function of proteins involved in transmission or pathogenesis, or through indirect effects, such as antibody dependent enhancement [68], which could lead to increases in viral load and ultimately greater disease severity.

Vaccines have the ability to provide protection against COVID-19 disease at a massive scale, but widespread protection could impose significant selection pressures on the SARS-CoV-2 virus. Current evidence suggests no immediate cause for concern about virulence evolution, but careful monitoring and efficacy evaluation are needed moving forward.

Methods

Epidemiological model

To evaluate the potential evolutionary consequences associated with SARS-CoV-2 vaccine candidates, we develop a compartmental ordinary differential equation model that broadly reflects SARS-CoV-2 epidemiology and the protective effects of naturally acquired and vaccine-induced immunity (Fig. 2). We separate the impacts of vaccinal and natural immunity in the upper and lower respiratory tracts, and the impact of these protective effects on infection, transmission, and virulence. We assume that infection in the LRT and URT additively contributes to transmission rate, β (Eqn. 1). The parameter ε defines the fractional contribution of the LRT to transmission. Transmission rates in both the URT and LRT follow an increasing and saturating function of virulence defined by shape parameters b_1 and b_2 . We assume that a virulence rate greater than 0.0025 is necessary for transmission (Eqn. 1). Immunity limits transmission by reducing virulence by a scalar $(1 - r_{U,V})$ in the upper respiratory system, and $(1 - r_{L,V})$ in the lower respiratory tracts (in this case transmission is calculated as $\beta(\alpha, r_{U,V}, r_{L,V})$). Naturally acquired immunity and a combination of naturally acquired and vaccinal immunity limit transmission in the same ways according to the parameters $r_{U,C}, r_{L,C}$ and $r_{U,CV}, r_{L,CV}$ respectively.

$$\beta(\alpha, r_U, r_L) = (1 - \varepsilon)b_1((1 - r_U)(\alpha - 0.0025))^{b_2} + \varepsilon b_1((1 - r_L)(\alpha - 0.0025))^{b_2} \quad (1)$$

Moving from how immunity affects transmission to considering how it might affect infection and virulence, we begin by connecting virulence to the rate of removal of infectious

individuals from the transmission pool. We set this rate equal to virulence (α) times a constant (p). This association between virulence and the removal of infectious individuals combined with the form of the function relating virulence to transmission constitutes a virulence-transmission trade-off (Fig. 1). To incorporate the effects of immunity on virulence, we assume that only LRT protection reduces virulence, in line with current reporting on the pathology of SARS-CoV-2. The effects of virulence on disease associated removal are reduced by a scalar $(1 - r_{L,V})$ for vaccinated individuals (in class V), by $(1 - r_{L,C})$ for convalescent individuals with naturally acquired immunity (in class C), and by $(1 - r_{L,C_V})$ for individuals with naturally acquired and vaccinal immunity (in class C_V). To model effects of immunity on transmission, we assume that the force of infection (λ) is reduced by a scalar $(1 - r_{U,V})$ for individuals in class V , by $(1 - r_{U,C})$ for individuals in class C , and by $(1 - r_{U,C_V})$ for individuals in class C_V , as the URT seems to be the key driver of transmission. Note that convalescent individuals (in both the C and C_V classes) can become reinfected if immunity provides incomplete protection against reinfection ($r_{U,C}$ or $r_{U,C_V} < 1$).

This framework for modeling the effects of immunity is designed to capture key differences between URT and LRT protection. To consider how vaccinal and natural immunity affect infection, transmission, and virulence without separating the effects of immunity between respiratory compartments, this framework would need to be adjusted to decouple reductions in transmission from reductions in infection and virulence.

After defining the relationships between immunity, transmission, infection, and virulence, we incorporate them into a compartmental epidemiological model (Fig. 2B, Eqn. 1-3). We assume a one day time step, and accordingly set the recovery rate $\gamma = \frac{1}{7}$ [69]. We ignore disease-associated mortality, and assume that isolation occurs before death. This is consistent

with the observation that death usually occurs several weeks after the appearance of symptoms. We assume that after individuals are isolated, they enter a quarantined class (Q_V for those individuals did have vaccinal immunity prior to infection, and Q for individuals who did not) in which they can neither contribute to transmission nor be reinfected. Individuals move from quarantined classes to convalescent classes (either C or C_V) at rate $q = \frac{1}{10}$ as they recover from infection and no longer contribute to transmission. Immunity wanes at rate ω for the convalescent class (C), and at rate ω_V for the vaccinated (V) and vaccinated-convalescent class (C_V). We ignore birth and death processes to hold the population size constant.

$$\lambda = \beta(\alpha, 0, 0) I_0 + \beta(\alpha, r_{U,V}, r_{L,V}) I_V + \beta(\alpha, r_{U,C}, r_{L,C}) I_C + \beta(\alpha, r_{U,C_V}, r_{L,C_V}) I_{C_V} \quad (2)$$

$$\frac{dS}{dt} = -S (\lambda + \mu) + C\omega + C_V\omega_V \quad (3a)$$

$$\frac{dV}{dt} = -V ((1 - r_{U,V}) \lambda) \quad (3b)$$

$$\frac{dI_0}{dt} = S \lambda - I_0 (\gamma + \alpha p) \quad (3c)$$

$$\frac{dI_V}{dt} = V (1 - r_{U,V}) \lambda - I_V (\gamma + (1 - r_{L,V}) \alpha p) \quad (3d)$$

$$\frac{dI_C}{dt} = C (1 - r_{U,C}) \lambda - I_C (\gamma + (1 - r_{L,C}) \alpha p) \quad (3e)$$

$$\frac{dI_{C_V}}{dt} = C_V (1 - r_{U,C_V}) \lambda - I_{C_V} (\gamma + (1 - r_{L,C_V}) \alpha p) \quad (3f)$$

$$\frac{dQ}{dt} = I_0 \alpha p + I_C (1 - r_{L,C}) \alpha p - Qq \quad (3g)$$

$$\frac{dQ_V}{dt} = I_V(1 - r_{L,V})\alpha p + I_{C_V}(1 - r_{L,C_V})\alpha p - Q_V q \quad (3h)$$

$$\frac{dC}{dt} = I_0\gamma + I_C\gamma + Qq - C((1 - r_{U,C})\lambda + \omega) \quad (3i)$$

$$\frac{dC_V}{dt} = I_V\gamma + I_{C_V}\gamma + Q_Vq - C_V((1 - r_{U,C_V})\lambda + \omega_V) \quad (3j)$$

Evolutionary analysis

The dynamics of the above model hinge on the relationship between transmission and virulence, and as such are sensitive to the shape of the virulence-transmission trade-off. We aim explore how the epidemiological and evolutionary outcomes predicted by this model change with assumptions about the effects of vaccinal immunity and where the virulence of the *delta* variant falls on the trade-off curve. We begin by defining the observed values of virulence associated with the ancestral strain and evolved variants. Building from an estimated infection fatality rate of the ancestral strain (α_{ansc}) of 0.005 [70], we set $\alpha_{alpha} = 1.5 * \alpha_{ansc} = 0.0075$. We assume the *delta* variant to be twice as virulent as the ancestral strain and set $\alpha_{delta} = 2 * \alpha_{ansc} = 0.01$.

Next, we define values of R_0 across COVID-19 strains. The transmission rate of *delta* is estimated to be approximately 1.5 times greater than that of the *alpha* variant [71], which in turn was estimated to be approximately 1.5 times greater than that of the ancestral strain. Given the basic reproduction number of the ancestral strain $R_{0ansc} = 2.5$ [72–74], we set $R_{0alpha} = 1.5 * R_{0ansc} = 3.75$ and $R_{0delta} = 1.5 * R_{0alpha} = 5.625$. Following this, we set p , the constant relating virulence to the rate of removal, to 50 so that the average amount of time an individual

(infected with the *delta* strain) is infectious before isolation is $\frac{1}{\alpha_{delta} * p} = 2$ days if recovery is ignored. This value is roughly consistent with the assumptions of other models [75].

Following this, we construct a set of optimal virulence (α_{optim}) values to explore that reflects various assumptions about the fitness of α_{delta} relative the optimum. Because the *delta* strain is assumed to be more fit than the *alpha* strain (i.e. it has a higher R_0), and the *alpha* strain more fit than the ancestral strain, we assume that the optimum virulence is either intermediate between α_{alpha} and α_{delta} , equal to α_{delta} , or greater than and α_{delta} : $\alpha_{optim} \in \{\frac{\alpha_{alpha} + \alpha_{delta}}{2}, \alpha_{delta}, 2 * \alpha_{delta}\}$. Figure S1 illustrates how this set of assumptions corresponds to different sets of relationships between virulence, transmission, transmission time, and fitness.

We use the next-generation matrix approach [76] to derive the following equation for the effective reproductive number R :

$$F = \begin{bmatrix} S*\beta(\alpha,0,0,\epsilon) & S*\beta(\alpha,r_{U,V},r_{L,V},\epsilon) & S*\beta(\alpha,r_{U,C},r_{L,C},\epsilon) & S*\beta(\alpha,r_{U,C_V},r_{L,C_V},\epsilon) & 0 & 0 \\ V*(1-r_{U,V})*\beta(\alpha,0,0,\epsilon) & V*(1-r_{U,V})*\beta(\alpha,r_{U,V},r_{L,V},\epsilon) & V*(1-r_{U,V})*\beta(\alpha,r_{U,C},r_{L,C},\epsilon) & V*(1-r_{U,V})*\beta(\alpha,r_{U,C_V},r_{L,C_V},\epsilon) & 0 & 0 \\ C*(1-r_{U,C})*\beta(\alpha,0,0,\epsilon) & C*(1-r_{U,C})*\beta(\alpha,r_{U,V},r_{L,V},\epsilon) & C*(1-r_{U,C})*\beta(\alpha,r_{U,C},r_{L,C},\epsilon) & C*(1-r_{U,C})*\beta(\alpha,r_{U,C_V},r_{L,C_V},\epsilon) & 0 & 0 \\ C_V*(1-r_{U,C_V})*\beta(\alpha,0,0,\epsilon) & C_V*(1-r_{U,C_V})*\beta(\alpha,r_{U,V},r_{L,V},\epsilon) & C_V*(1-r_{U,C_V})*\beta(\alpha,r_{U,C},r_{L,C},\epsilon) & C_V*(1-r_{U,C_V})*\beta(\alpha,r_{U,C_V},r_{L,C_V},\epsilon) & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix} \quad 4(a)$$

$$V = \begin{bmatrix} \gamma + \alpha p & 0 & 0 & 0 & 0 & 0 \\ 0 & \gamma + (1 - r_{L,V}) \alpha p & 0 & 0 & 0 & 0 \\ 0 & 0 & \gamma + (1 - r_{L,C}) \alpha p & 0 & 0 & 0 \\ 0 & 0 & 0 & \gamma + (1 - r_{L,C_V}) \alpha p & 0 & 0 \\ -\alpha p & 0 & -(1 - r_{L,C}) \alpha p & 0 & q & 0 \\ 0 & -(1 - r_{L,V}) \alpha p & 0 & -(1 - r_{L,C_V}) \alpha p & 0 & q \end{bmatrix} \quad 4(b)$$

$$R = \text{The spectral radius of } F * V^{-1} \quad 4(c)$$

When evaluated for a completely susceptible population, equation 4 gives R_0 , the basic reproductive number. For a given assumption about the value of α_{optim} , we used equation 4 and numerical methods to set the value of b_2 to that which maximizes R_0 at α_{optim} and $b_1=1$, and the value of b_1 to that which corresponded $R_0 = R_{0_delta}$ at α_{delta} . We checked to ensure that the value of b_2 was insensitive to the value of b_1 , that α_{optim} maximized R_0 , and that α_{delta} correspond to $R_0 = R_{0_delta}$ at the identified values of b_1 and b_2 . Figure 1 shows the virulence transmission trade-off curves constructed from the three assumptions about the value of α_{optim} ; these assumptions map to a wide range of possible shapes of the trade-off.

To characterize the outcome of pathogen virulence evolution in response to vaccination we identify how a range of vaccinal protection characteristics in the URT and LRT map to epidemiological outcomes before pathogen evolution occurs, as well as patterns of virulence after many iterative virulence evolution events. Because SARS-CoV-2 virulence evolution has been observed to play out over a matter of months, we ignore processes that operate on longer time scales such as births and deaths. Additionally, we set $\omega = 0$ and $\omega_V = 0$ so that immunity does not wane. While immunological waning certainly does occur, its effects are likely small on the timescale of virulence evolution. In the adaptive dynamics approach that we employ (see below), incremental changes in pathogen virulence are assumed to occur only after epidemiological equilibrium is reached. Including immunological waning would force the model to take many years to reach equilibrium, making our predictions fall significantly out of step with the observed rate of pathogen evolution. An alternate way to incorporate the effects of immunological waning into our analyses is to consider the values of the parameters $r_{U,V}$ and $r_{L,V}$ to reflect mean population values that account for variation in vaccinal immunity across individuals. As such, the effects of immunological waning could be conceptualized as decreasing

the values of $r_{U,V}$ and $r_{L,V}$. Likewise, booster vaccine doses could be conceptualized as increasing the values of these two parameters.

We iterate our analysis for various scenarios encompassing different vaccination rates and values of α_{optim} . We only consider values of $r_{U,V}$ and $r_{L,V}$ in $[0.5, 1.0]$ as all widely used vaccines have been shown to be at least 50% effective in preventing infection and severe disease [77]. In the scenarios presented in the main text, we set $r_{U,C} = 0.5$ and $r_{L,C} = 0.75$. Figures S3-S5 give results for alternate scenarios in which we consider stronger ($r_{U,C} = 0.75$ and $r_{L,C} = 0.9$) and weaker ($r_{U,C} = 0.25$ and $r_{L,C} = 0.5$) effects of natural immunity. Additionally, because vaccinated individuals who experience breakthrough infections exhibit immunity stronger than either vaccinal or natural immunity alone [78], we set $r_{U,CV} = \frac{1+r_{U,V}}{2}$ and $r_{L,CV} = \frac{1+r_{L,V}}{2}$.

For each unique combination of $r_{U,V}$, $r_{L,V}$, α_{optim} , and vaccination rate (v), we first initialize the model assuming that 37.5% of individuals are convalescent (roughly consistent with the estimated cumulative incidence in the U.S. as of May 2021 [79]), that 0.1% of individuals are infected, and that a fraction v (with $v \leq 1 - 0.001$) are vaccinated. Using these assumptions, we set the initial conditions as follows:

$$S(0) = 1 - C_V(0) - C(0) - V(0) - 0.001 \quad (5a)$$

$$V(0) = (1 - 0.375) * v \quad (5b)$$

$$I(0) = 0.001 * \frac{S(0)}{C_V(0) + C(0) + V(0) + S(0)} \quad (5c)$$

$$I_V(0) = 0.001 * \frac{V(0)}{C_V(0) + C(0) + V(0) + S(0)} \quad (5d)$$

$$I_C(0) = 0.001 * \frac{C(0)}{C_V(0) + C(0) + V(0) + S(0)} \quad (5e)$$

$$I_{CV}(0) = 0.001 * \frac{C_V(0)}{C_V(0) + C(0) + V(0) + S(0)} \quad (5f)$$

$$Q(0) = 0 \quad (5g)$$

$$Q_V(0) = 0 \quad (5h)$$

$$C(0) = 0.375 * (1 - \nu) \quad (5i)$$

$$C_V(0) = 0.375 * \nu \quad (5j)$$

Next, we calculate the effective reproductive number (R_E) for α_{delta} by evaluating equation 4 at these starting conditions. R_E values are informative about the short term epidemiological consequences of vaccination before pathogen evolution acts. Values less than one indicate that herd immunity is achieved (within the theoretical population in consideration), while values greater than or equal to one indicate that this is not the case.

After calculating R_E for α_{delta} at the starting conditions, we proceed with an adaptive dynamics approach to identify the evolutionarily stable virulence strategy (α_{ES}) associated with the $r_{U,V}$, $r_{L,V}$, α_{optim} , and ν parameters. Evolutionarily stable virulence strategies can resist invasion by any other strategy (i.e. $R_E < 1$ for $\alpha \neq \alpha_{ES}$), and as a result are expected to be the equilibrium strategy over evolutionary time. We consider potential virulence strategies in the set $A = \{ \{0.0025, 0.0030, 0.0035, \dots, 0.0500\}, \{0.055, 0.06, 0.065, \dots, 0.4000\} \}$. For each $\alpha_{resident} \in A$, we 1) set $\alpha = \alpha_{resident}$ and simulate epidemiological dynamics over 1 year using the R package ‘desolve’ [80] and the ‘lsoda’ integrator with a step size of one day, 2) extract frequencies of individuals in each class as the epidemiological equilibrium conditions, and finally 3) for each $\alpha_{invader} \in A$, set $\alpha = \alpha_{invader}$ and calculate R_E by evaluating equation 4 at the epidemiological equilibrium conditions. We then plot the ability of each $\alpha_{invader}$ strategy to

invade each $\alpha_{resident}$ strategy (i.e. $R_E > 1$) to generate pairwise invasibility plots, and use these plots to identify evolutionarily stable strategies (fig. S2).

Code availability

All code used in the analyses is available on GitHub [81].

Supplementary figures

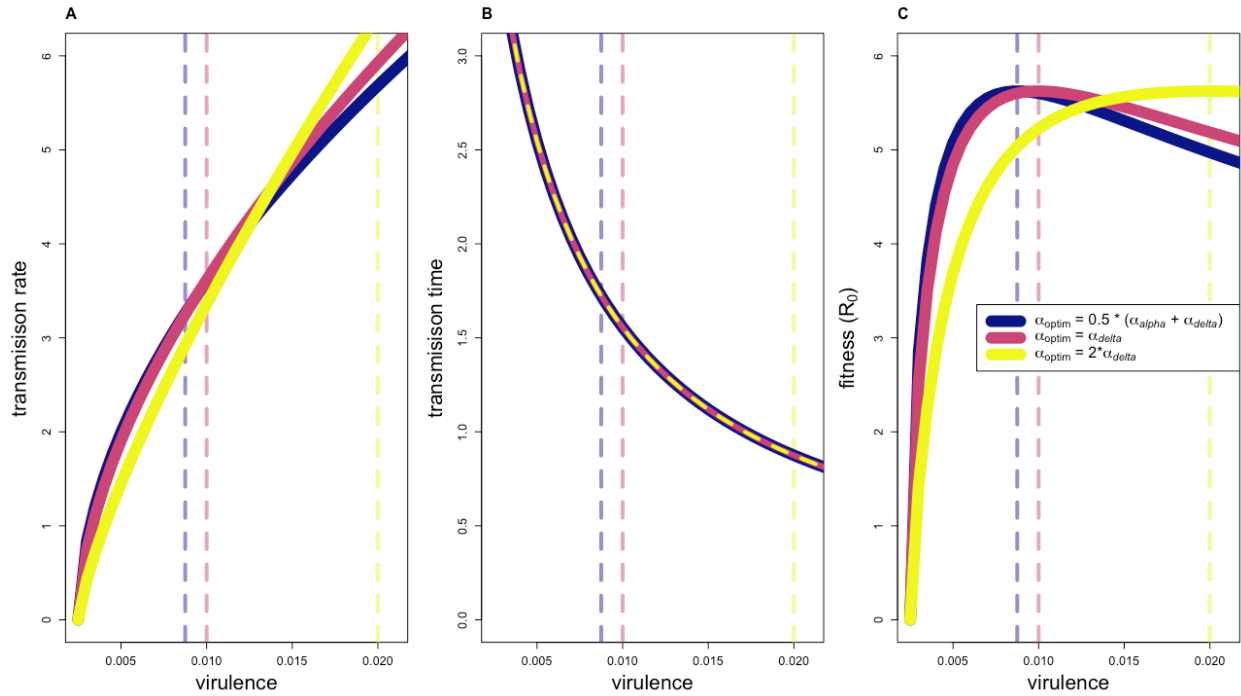


Figure S1: Optimum virulence scenarios. We consider three scenarios for the value of α_{optim} : 1) α_{optim} is intermediate between α_{alpha} and α_{delta} (purple lines), 2) α_{optim} is equal to α_{delta} (pink lines), and 3) α_{optim} is two times α_{delta} (yellow lines). For each of these scenarios, panel A shows the relationship between virulence and transmission rate, panel B shows the relationship between virulence and transmission time, and panel C shows the relationship between virulence and fitness (in a completely susceptible population, equivalent to R_0). Vertical dashed lines indicate the optimum virulence values for each scenario.

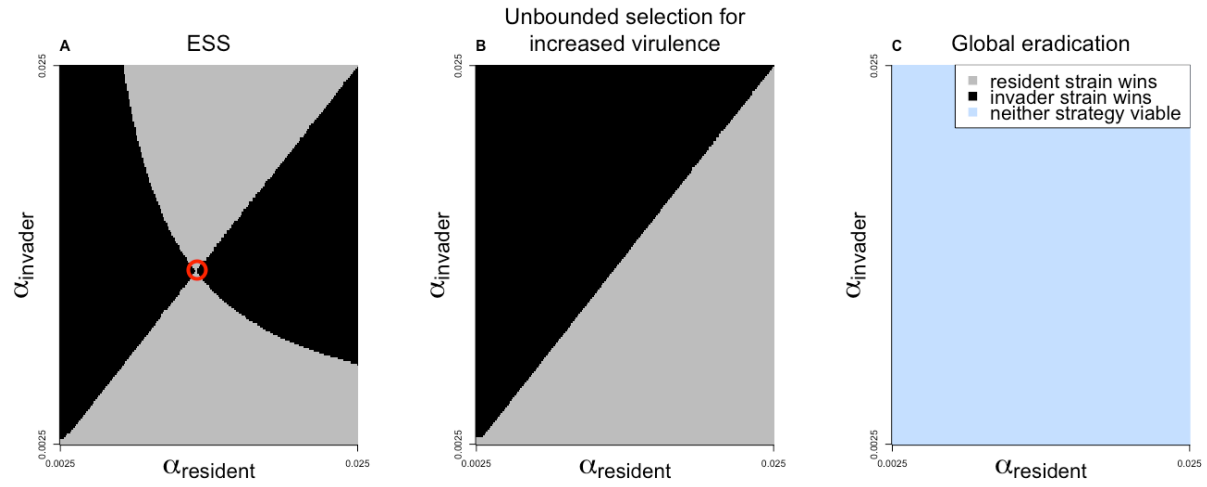


Figure S2: Pairwise invasibility plot motifs. We differentiated between three pairwise invasibility plot motifs during our adaptive dynamics analyses. Panel A shows a motif corresponding to an evolutionarily stable virulence strategy. The red circle indicates that this strategy corresponds to the $\alpha_{resident}$ value that can resist invasion by any strategy other than its own. Panel B shows a motif corresponding to unbounded selection for increased virulence. In this motif, all values of $\alpha_{resident}$ can be invaded by a strain with $\alpha_{invader} > \alpha_{resident}$, indicating that selection for increased virulence will proceed without bounds. Panel C shows a motif corresponding to evolution-proof herd immunity. No virulence strategies are viable.

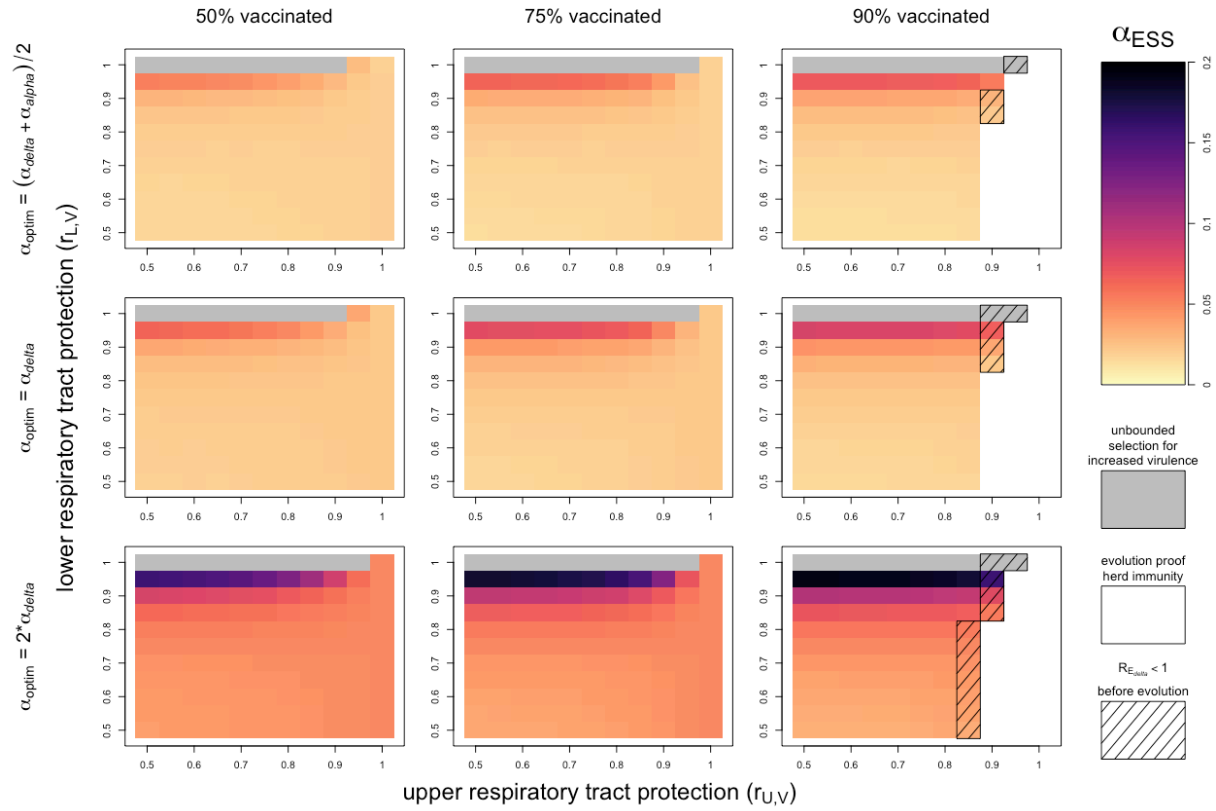


Figure S3: Virulence evolution and evo-epidemiological outcomes associated with vaccinal immunity when LRT infection contributes minimally to transmission. We found that patterns of virulence evolution and evo-epidemiological consequences are qualitatively similar when infection in the URT and LRT contribute equally to transmission ($\varepsilon = 0.5$, Fig. 3), and when LRT infection contributes minimally to transmission ($\varepsilon = 0.1$, this figure). Panels show the evolutionarily stable virulence strategy resulting from different combinations of vaccinal protection in the LRT and URT as in figure 3. In all panels $r_{U,C} = 0.5$, $r_{L,C} = 0.75$.

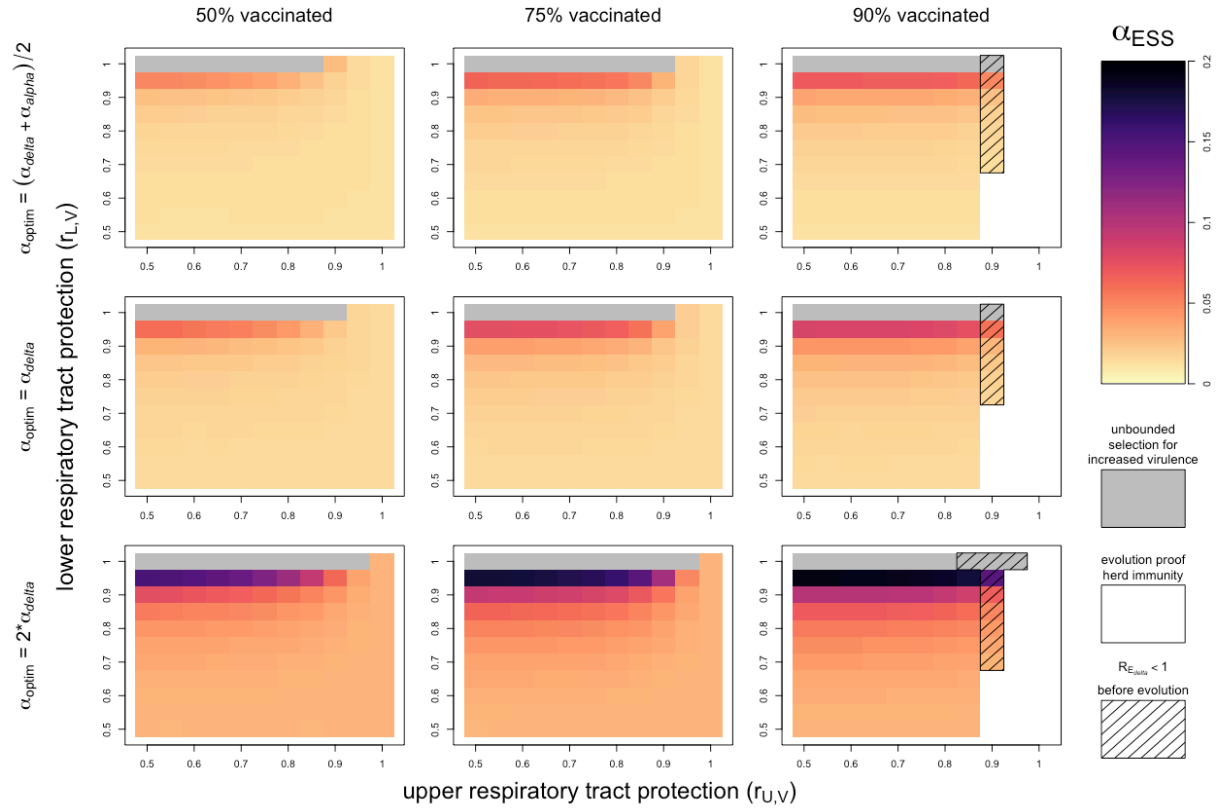


Figure S4: Virulence evolution and evo-epidemiological outcomes associated with vaccinal immunity with weak effects of natural immunity. Our analyses returned qualitatively similar results across a range of assumptions about the strength of natural immunity. This figure shows results for weaker assumed effects of naturally acquired immunity ($r_{U,C} = 0.25$, $r_{L,C} = 0.5$) than in the analyses presented in the main text ($r_{U,C} = 0.5$, $r_{L,C} = 0.75$, Fig. 3). Panels show the evolutionarily stable virulence strategy resulting from different combinations of vaccinal protection in the LRT and URT as in figure 3. The lower and upper respiratory tracts contribute equally to transmission ($\varepsilon = 0.5$).

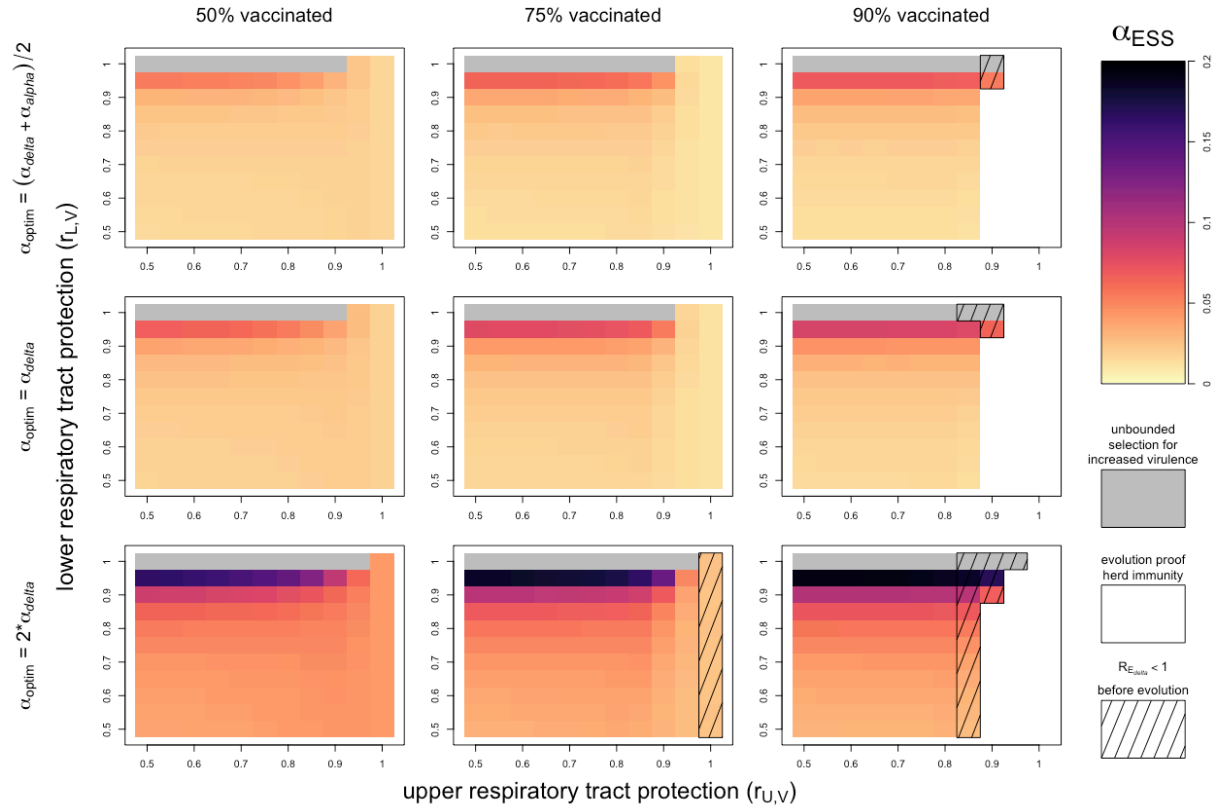


Figure S5: Virulence evolution and evo-epidemiological outcomes associated with vaccinal immunity with strong effects of natural immunity. Our analyses returned qualitatively similar results across a range of assumptions about the strength of natural immunity. This figure shows results for stronger assumed effects of naturally acquired immunity ($r_{U,C} = 0.75, r_{L,C} = 0.75$) than in the analyses presented in the main text ($r_{U,C} = 0.5, r_{L,C} = 0.75$, Fig. 3). Panels show the evolutionarily stable virulence strategy resulting from different combinations of vaccinal protection in the LRT and URT as in figure 3. The lower and upper respiratory tracts equally contribute to transmission ($\varepsilon = 0.5$).

References

1. World Health Organization. 2021 COVID-19 Weekly Epidemiological Update 63.
2. World Bank. 2020 Global Economic Prospects, June 2020.
3. Polack FP *et al.* 2020 Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N. Engl. J. Med.* **383**, 2603–2615. (doi:10.1056/NEJMoa2034577)
4. Baden LR *et al.* 2020 Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N. Engl. J. Med.* **384**, 403–416. (doi:10.1056/NEJMoa2035389)
5. Voysey M *et al.* 2021 Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* **397**, 99–111. (doi:10.1016/S0140-6736(20)32661-1)
6. Logunov DY *et al.* 2021 Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia. *Lancet* (doi:10.1016/S0140-6736(21)00234-8)
7. Kennedy DA, Read AF. 2020 Monitor for COVID-19 vaccine resistance evolution during clinical trials. *PLOS Biol.* **18**, e3001000. (doi:10.1371/journal.pbio.3001000)
8. Fisman DN, Tuite AR. 2021 Evaluation of the relative virulence of novel SARS-CoV-2 variants: a retrospective cohort study in Ontario, Canada. *Can. Med. Assoc. J.* **193**, E1619–E1625. (doi:10.1503/cmaj.211248)
9. Delahoy MJ *et al.* 2021 Hospitalizations Associated with COVID-19 Among Children and Adolescents - COVID-NET, 14 States, March 1, 2020–August 14, 2021. *MMWR. Morb. Mortal. Wkly. Rep.* **70**, 1255–1260. (doi:10.15585/mmwr.mm7036e2)
10. Taylor CA *et al.* 2021 Severity of Disease Among Adults Hospitalized with Laboratory-Confirmed COVID-19 Before and During the Period of SARS-CoV-2 B.1.617.2 (Delta)

- Predominance - COVID-NET, 14 States, January-August 2021. *MMWR. Morb. Mortal. Wkly. Rep.* **70**, 1513–1519. (doi:10.15585/mmwr.mm7043e1)
11. Levin S, Pimentel D. 1981 Selection of Intermediate Rates of Increase in Parasite-Host Systems. *Am. Nat.* **117**, 308–315. (doi:10.1086/283708)
 12. Anderson RM, May RM. 1982 Coevolution of hosts and parasites. *Parasitology* **85**, 411–426. (doi:10.1017/S0031182000055360)
 13. Alizon S, Hurford A, Mideo N, van Baalen M. 2009 Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. *J. Evol. Biol.* **22**, 245–259. (doi:10.1111/j.1420-9101.2008.01658.x)
 14. Bull JJ, Llaurens A. 2014 Theory and Empiricism in Virulence Evolution. *PLoS Pathog.* **10**, e1004387. (doi:10.1371/journal.ppat.1004387)
 15. Bull JJ, Badgett MR, Springman R, Molineux IJ. 2004 Genome properties and the limits of adaptation in bacteriophages. *Evolution (N. Y.)*. **58**, 692–701. (doi:10.1111/j.0014-3820.2004.tb00402.x)
 16. Osnas EE, Dobson AP. 2010 Evolution of virulence when transmission occurs before disease. *Biol. Lett.* **6**, 505–508. (doi:10.1098/rsbl.2009.1019)
 17. Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C, Sagulenko P, Bedford T, Neher RA. 2018 Nextstrain: real-time tracking of pathogen evolution. *Bioinformatics* **34**, 4121–4123. (doi:10.1093/bioinformatics/bty407)
 18. Nextstrain. In press. Genomic epidemiology of novel coronavirus - Global subsampling. See <https://nextstrain.org/ncov/gisaid/global> (accessed on 11 January 2021).
 19. World Health Organization. In press. Tracking SARS-CoV-2 variants.
 20. Davies NG *et al.* 2021 Estimated transmissibility and impact of SARS-CoV-2 lineage

- B.1.1.7 in England. *Science* **372**, eabg3055. (doi:10.1126/science.abg3055)
21. Alizon S, Haim-Boukobza S, Foulongne V, Verdurme L, Trombert-Paolantoni, Lecorche E, Roquebert B, Sofonea MT. 2021 Rapid spread of the SARS-CoV-2 Delta variant in some French regions, June 2021. *Eurosurveillance* **26**.
 22. Public Health England. 2021 SARS-CoV-2 variants of concern and variants under investigation in England Technical briefing 15.
 23. Yurkovetskiy L *et al.* 2020 Structural and Functional Analysis of the D614G SARS-CoV-2 Spike Protein Variant. *Cell* **183**, 739-751.e8. (doi:10.1016/j.cell.2020.09.032)
 24. Korber B *et al.* 2020 Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. *Cell* **182**, 812-827.e19. (doi:10.1016/j.cell.2020.06.043)
 25. Plante JA *et al.* 2020 Spike mutation D614G alters SARS-CoV-2 fitness. *Nature* (doi:10.1038/s41586-020-2895-3)
 26. Lorenzo-Redondo R *et al.* 2020 A clade of SARS-CoV-2 viruses associated with lower viral loads in patient upper airways. *EBioMedicine* **62**, 103112. (doi:10.1016/j.ebiom.2020.103112)
 27. Davies NG *et al.* 2021 Increased mortality in community-tested cases of SARS-CoV-2 lineage B.1.1.7. *Nature* (doi:10.1038/s41586-021-03426-1)
 28. Twohig KA *et al.* 2021 Hospital admission and emergency care attendance risk for SARS-CoV-2 delta (B. 1.617. 2) compared with alpha (B. 1.1. 7) variants of concern: a cohort study. *Lancet Infect. Dis.*
 29. Volz E *et al.* 2020 Evaluating the Effects of SARS-CoV-2 Spike Mutation D614G on Transmissibility and Pathogenicity. *Cell* (doi:https://doi.org/10.1016/j.cell.2020.11.020)

30. Alizon S, Sofonea MT. 2021 SARS-CoV-2 virulence evolution: Avirulence theory, immunity and trade-offs. *J. Evol. Biol.* **n/a**. (doi:<https://doi.org/10.1111/jeb.13896>)
31. Otto SP *et al.* 2021 The origins and potential future of SARS-CoV-2 variants of concern in the evolving COVID-19 pandemic. *Curr. Biol.* **31**, R918–R929. (doi:<https://doi.org/10.1016/j.cub.2021.06.049>)
32. Ferretti L *et al.* 2020 The timing of COVID-19 transmission. *medRxiv* , 2020.09.04.20188516. (doi:[10.1101/2020.09.04.20188516](https://doi.org/10.1101/2020.09.04.20188516))
33. Wu Z, McGoogan JM. 2020 Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. *Jama* **2019**, 3–6. (doi:[10.1001/jama.2020.2648](https://doi.org/10.1001/jama.2020.2648))
34. Zhou F *et al.* 2020 Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* **395**, 1054–1062. (doi:[https://doi.org/10.1016/S0140-6736\(20\)30566-3](https://doi.org/10.1016/S0140-6736(20)30566-3))
35. Graham MS *et al.* 2021 Changes in symptomatology, reinfection, and transmissibility associated with the SARS-CoV-2 variant B.1.1.7: an ecological study. *Lancet Public Heal.* **6**, e335–e345. (doi:[https://doi.org/10.1016/S2468-2667\(21\)00055-4](https://doi.org/10.1016/S2468-2667(21)00055-4))
36. Ali ST, Wang L, Lau EHY, Xu X-K, Du Z, Wu Y, Leung GM, Cowling BJ. 2020 Serial interval of SARS-CoV-2 was shortened over time by nonpharmaceutical interventions. *Science* **369**, 1106 LP – 1109. (doi:[10.1126/science.abc9004](https://doi.org/10.1126/science.abc9004))
37. Day T, Gandon S, Lion S, Otto SP. 2020 On the evolutionary epidemiology of SARS-CoV-2. *Curr. Biol.* **30**, R849–R857. (doi:[10.1016/j.cub.2020.06.031](https://doi.org/10.1016/j.cub.2020.06.031))
38. Gandon S, Mackinnon MJ, Nee S, Read AF. 2001 Imperfect vaccines and the evolution of

- pathogen virulence. *Nature* **414**, 751–756. (doi:10.1038/414751a)
39. Miller IF, Metcalf CJ. 2019 Vaccine-driven virulence evolution: consequences of unbalanced reductions in mortality and transmission and implications for pertussis vaccines. *J. R. Soc. Interface* **16**, 20190642. (doi:10.1098/rsif.2019.0642)
 40. Mitchell PK, Lipsitch M, Hanage WP. 2015 Carriage burden, multiple colonization and antibiotic pressure promote emergence of resistant vaccine escape pneumococci. *Philos. Trans. R. Soc. B Biol. Sci.* **370**, 20140342. (doi:10.1098/rstb.2014.0342)
 41. R. MF, Hans van O, Kees H, J. van der HHG, Wim G, L. WRJ. 1998 Polymorphism in the Bordetella pertussis Virulence Factors P.69/Pertactin and Pertussis Toxin in The Netherlands: Temporal Trends and Evidence for Vaccine-Driven Evolution. *Infect. Immun.* **66**, 670–675. (doi:10.1128/IAI.66.2.670-675.1998)
 42. Gandon S, Day T. 2008 Evidences of parasite evolution after vaccination. *Vaccine* **26**, 23–26. (doi:10.1016/j.vaccine.2008.02.007)
 43. Mackinnon MJ, Read AF. 2004 Immunity Promotes Virulence Evolution in a Malaria Model. *PLoS Biol.* **2**, e230. (doi:10.1371/journal.pbio.0020230)
 44. Read AF, Baigent SJ, Powers C, Kgosana LB, Blackwell L, Smith LP, Kennedy DA, Walkden-Brown SW, Nair VK. 2015 Imperfect vaccination can enhance the transmission of highly virulent pathogens. *PLoS Biol.* **13**, 1–18. (doi:10.1371/journal.pbio.1002198)
 45. Krammer F. 2020 SARS-CoV-2 vaccines in development. *Nature* **586**, 516–527. (doi:10.1038/s41586-020-2798-3)
 46. Kuiken T, Riteau B, Fouchier R, Rimmelzwaan G. 2012 Pathogenesis of influenza virus infections: the good, the bad and the ugly. *Curr. Opin. Virol.* **2**, 276–286. (doi:10.1016/j.coviro.2012.02.013)

47. Reperant LA, Kuiken T, Grenfell BT, Osterhaus ADME, Dobson AP. 2012 Linking Influenza Virus Tissue Tropism to Population-Level Reproductive Fitness. *PLoS One* **7**, e43115. (doi:10.1371/journal.pone.0043115)
48. Hou YJ *et al.* 2020 SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract. *Cell* **182**, 429-446.e14. (doi:10.1016/j.cell.2020.05.042)
49. Muñoz-Fontela C *et al.* 2020 Animal models for COVID-19. *Nature* **586**, 509–515. (doi:10.1038/s41586-020-2787-6)
50. Mitrani RD, Dabas N, Goldberger JJ. 2020 COVID-19 cardiac injury : Implications for long-term surveillance and outcomes in survivors. *Heart. Rhythm* **17**, 1984–1990. (doi:10.1016/j.hrthm.2020.06.026)
51. Chandrashekar A *et al.* 2020 SARS-CoV-2 infection protects against rechallenge in rhesus macaques. *Science* **369**, 812–817. (doi:10.1126/science.abc4776)
52. Lumley SF *et al.* 2020 Antibodies to SARS-CoV-2 are associated with protection against reinfection. *medRxiv* , 2020.11.18.20234369. (doi:10.1101/2020.11.18.20234369 ;)
53. Nasreen S *et al.* 2021 Effectiveness of mRNA and ChAdOx1 COVID-19 vaccines against symptomatic SARS-CoV-2 infection and severe outcomes with variants of concern in Ontario. *medRxiv* , 2021.06.28.21259420. (doi:10.1101/2021.06.28.21259420)
54. Voysey M *et al.* 2021 Single dose administration, and the influence of the timing of the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine. *SSRN*
55. Haas EJ *et al.* 2021 Impact and effectiveness of mRNA BNT162b2 vaccine against SARS-CoV-2 infections and COVID-19 cases, hospitalisations, and deaths following a nationwide vaccination campaign in Israel: an observational study using national

- surveillance data. *Lancet* **397**, 1819–1829. (doi:10.1016/S0140-6736(21)00947-8)
56. Lopez Bernal J *et al.* 2021 Effectiveness of Covid-19 Vaccines against the B.1.617.2 (Delta) Variant. *N. Engl. J. Med.* **385**, 585–594. (doi:10.1056/NEJMoa2108891)
 57. Mizrahi B, Lotan R, Kalkstein N, Peretz A, Perez G, Ben-Tov A, Chodick G, Gazit S, Patalon T. 2021 Correlation of SARS-CoV-2 Breakthrough Infections to Time-from-vaccine; Preliminary Study. *medRxiv* , 2021.07.29.21261317. (doi:10.1101/2021.07.29.21261317)
 58. Goldberg Y *et al.* 2021 Waning immunity of the BNT162b2 vaccine: A nationwide study from Israel. *medRxiv* , 2021.08.24.21262423. (doi:10.1101/2021.08.24.21262423)
 59. Bar-On YM *et al.* 2021 Protection of BNT162b2 Vaccine Booster against Covid-19 in Israel. *N. Engl. J. Med.* **385**, 1393–1400. (doi:10.1056/NEJMoa2114255)
 60. Wang Z *et al.* 2021 mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. *bioRxiv* , 2021.01.15.426911. (doi:10.1101/2021.01.15.426911)
 61. Ashby B, Best A. 2021 Herd immunity. *Curr. Biol.* **31**, R174–R177. (doi:https://doi.org/10.1016/j.cub.2021.01.006)
 62. Lavine JS, Bjornstad ON, Antia R. 2021 Immunological characteristics govern the transition of COVID-19 to endemicity. *Science* **371**, 741 LP – 745. (doi:10.1126/science.abe6522)
 63. Kemp SA *et al.* 2021 SARS-CoV-2 evolution during treatment of chronic infection. *Nature* **592**, 277–282. (doi:10.1038/s41586-021-03291-y)
 64. Van Dorp L, Tan CC, Lam SD, Richard D, Owen C, Berchtold D, Orengo C, Balloux F. 2020 Recurrent mutations in SARS-CoV-2 genomes isolated from mink point to rapid host-adaptation. *bioRxiv* (doi:10.1101/2020.11.16.384743)

65. Munnink BBO *et al.* 2020 Jumping back and forth : anthropozoonotic and zoonotic transmission of SARS-CoV-2 on mink farms. *medRxiv*
(doi:10.1101/2020.09.01.277152v1)
66. Saad-Roy CM *et al.* 2020 Immune life history, vaccination, and the dynamics of SARS-CoV-2 over the next 5 years. *Science* **370**, 811–818. (doi:10.1126/science.abd7343)
67. Kissler SM, Tedijanto C, Goldstein E, Grad YH, Lipsitch M. 2020 Projecting the transmission dynamics of SARS-CoV-2 through the postpandemic period. *Science* **368**, 860–868. (doi:10.1126/science.abb5793)
68. Arvin AM, Fink K, Schmid MA, Cathcart A, Spreafico R, Havenar-Daughton C, Lanzavecchia A, Corti D, Virgin HW. 2020 A perspective on potential antibody-dependent enhancement of SARS-CoV-2. *Nature* **584**, 353–363. (doi:10.1038/s41586-020-2538-8)
69. Bar-On YM, Flamholz A, Phillips R, Milo R. 2020 SARS-CoV-2 (COVID-19) by the numbers. *Elife* **9**, 1–15. (doi:10.7554/eLife.57309)
70. O’Driscoll M, Ribeiro Dos Santos G, Wang L, Cummings DAT, Azman AS, Paireau J, Fontanet A, Cauchemez S, Salje H. 2020 Age-specific mortality and immunity patterns of SARS-CoV-2. *Nature* (doi:10.1038/s41586-020-2918-0)
71. Allen H *et al.* 2021 Increased household transmission of COVID-19 cases associated with SARS-CoV-2 Variant of Concern B.1.617.2: a national case control study.
72. Park SW, Bolker BM, Champredon D, Earn DJD, Li M, Weitz JS, Grenfell BT, Dushoff J. 2020 Reconciling early-outbreak estimates of the basic reproductive number and its uncertainty: Framework and applications to the novel coronavirus (SARS-CoV-2)
outbreak: Reconciling early-outbreak estimates of the basic reproductive number and its

- uncertainty. *J. R. Soc. Interface* **17**. (doi:10.1098/rsif.2020.0144rsif20200144)
73. Liu Y, Gayle AA, Wilder-Smith A, Rocklöv J. 2020 The reproductive number of COVID-19 is higher compared to SARS coronavirus. *J. Travel Med.* , 1–4. (doi:10.1093/jtm/taaa021)
 74. Kucharski AJ *et al.* 2020 Early dynamics of transmission and control of COVID-19: a mathematical modelling study. *Lancet Infect. Dis.* **3099**, 1–7. (doi:10.1016/s1473-3099(20)30144-4)
 75. Kucharski AJ *et al.* 2020 Effectiveness of isolation, testing, contact tracing, and physical distancing on reducing transmission of SARS-CoV-2 in different settings: a mathematical modelling study. *Lancet Infect. Dis.* **20**, 1151–1160. (doi:10.1016/S1473-3099(20)30457-6)
 76. Diekmann O, Heesterbeek JAP, Metz JAJ. 1990 On the definition and the computation of the basic reproduction ratio R_0 in models for infectious diseases in heterogeneous populations. *J. Math. Biol. Math. Biol.* **28**, 365–382.
 77. Tregoning JS, Flight KE, Higham SL, Wang Z, Pierce BF. 2021 Progress of the COVID-19 vaccine effort: viruses, vaccines and variants versus efficacy, effectiveness and escape. *Nat. Rev. Immunol.* **21**, 626–636. (doi:10.1038/s41577-021-00592-1)
 78. Gazit S *et al.* 2021 Comparing SARS-CoV-2 natural immunity to vaccine-induced immunity: reinfections versus breakthrough infections. *medRxiv* , 2021.08.24.21262415. (doi:10.1101/2021.08.24.21262415)
 79. Centers for Disease Control and Prevention. 2021 Estimated COVID-19 Burden.
 80. Soetaert K, Petzoldt T, Setzer RW. 2010 Package deSolve : Solving Initial Value Differential Equations in R. *J. Stat. Softw.* **33**, 1–25. (doi:10.18637/jss.v033.i09)

81. Miller IF. 2021 Code for: No current evidence for risk of vaccine-driven virulence evolution in SARS-CoV-2.

https://github.com/ianfmiller/covid_vaccines_virulence_evolution

Chapter IV: Predicting the effects of climate change on the cross-scale epidemiological dynamics of a fungal plant pathogen

Foreword

This chapter is being prepared for submission to a peer reviewed journal:

Miller, I.F., Jiranek, J., Brownell, M., Coffey, S., Gray, B., Stahl, M., Metcalf, C.J.E. (in prep).

Predicting the effects of climate change on the cross-scale epidemiological dynamics of a fungal plant pathogen.

Abstract

The potential for climate change to exacerbate the burden of human infectious diseases is increasingly recognized, but its effects on infectious diseases of plants have received less attention. Understanding the impacts of climate on the epidemiological dynamics of plant pathogens is imperative, as these organisms play central roles in natural ecosystems and also pose a serious threat to agricultural production and food security. We use the fungal ‘flax rust’ pathogen (*Melampsora lini*) and its subalpine wildflower host Lewis flax (*Linum lewisii*) to investigate how climate change might affect the epidemiology of fungal plant pathogens. Our results suggest that climate change will initially slow transmission at both the within- and between-host scales. However, resurgences in disease spread are predicted as warming progresses, especially if the rate of greenhouse gas emissions continues to increase at its current pace. These findings represent an important step towards building a holistic understanding of climate effects on plant infectious disease that encompasses demographic, epidemiological, and evolutionary processes. A core result is that neglecting processes at any one scale of plant pathogen transmission may bias projections of climate effects, as climate drivers have variable and cascading impacts on processes underlying transmission that occur at different scales.

Introduction

Understanding the epidemiology of plant pathogens in a changing world is important, as these organisms significantly hinder agricultural production [1,2], threaten food security [3,4], and play a crucial role in mediating ecosystem processes [5,6]. Environmental conditions have long been known to be important determinants of disease spread in plant pathosystems [7], and climate change is increasingly recognized as a dominant driver of current and future trends [8]. However, there is a surprising knowledge gap surrounding the effects of climate on plant pathogen epidemiology, i.e., the dynamics of disease spread within populations.

Most previous studies of plant pathogen spread in the context of climate change have focused on disease severity, infection risk, or the geographic distribution of disease [9]. However, anticipating and mitigating the changing burden of pathogens on natural or agricultural plant populations requires an epidemiological perspective [10,11]. The impact of disease on the mortality, fecundity, biomass, or agricultural productivity of a population depends not only on the consequences of infection for individual plants, but also on the proportion of plants that become infected. Furthermore, the details of within- and between-host processes will shape future impacts infectious disease, particularly if climate change has cascading effects across scales of transmission. This could occur if, for example, warmer temperatures speed disease progression within infected plants resulting in increased transmission, and also increase the odds that a healthy plant becomes infected when challenged.

Of the few studies that have investigated effects of climate change on plant disease spread at the population scale, all (to our knowledge) have taken a statistical approach, directly extrapolating relationships between climate variables (e.g., temperature) and epidemiological variables (e.g., prevalence) to make projections. None have formally encompassed the

underlying within-host processes inherent to pathogen transmission, evaluated how these are affected by climate, and integrated across these within-host processes to generate a mechanistic (or semi-mechanistic) projection of the effects of climate on epidemiological variables like pathogen prevalence. While previous studies are tremendously useful for detecting broad scale effects of climate on disease dynamics [12,13], generating robust predictions requires epidemiological models that both capture climate effects on different scales of transmission and link these processes in one predictive framework. Such models are widely applied in the fields of human and animal infectious disease dynamics [14], but are underutilized in the realm of plant infectious disease, with a focus typically limited to disease severity [15].

In this study, we use *Linum lewisii*, a sub-alpine wildflower, and *Melampsora lini*, its fungal pathogen, as a model to investigate the effects of climate change on cross-scale transmission processes using a semi-mechanistic epidemiological model. This ‘flax rust’ system [16] is a representative example for the effects of climate change on the epidemiology of fungal plant pathogens, and also serves as an analogue for flax rust disease in agricultural systems. Our findings indicate that disease severity and prevalence could decrease in the short term, but eventually rebound due to continued climate change.

Results

Study system

We aim to predict how climate change might alter the within and between host transmission dynamics of fungal plant pathogens using the flax rust model system. The host, *L. lewisii* (Lewis flax), is a short-lived perennial plant distributed across the western United States, and the

pathogen, *M. lini*, is rust fungus. *M. lini* urediniospores infect the stems and leaves of *L. lewisii*, forming bright yellow-orange pustules (Fig. 1A). The spores propagated by these pustules are dispersed by the wind both within and between plants. This infection cycle repeats continuously over the course of a growing season [16]. The flax rust pathosystem is an excellent model for studying the effects of climate on the epidemiology of plant pathogens because *M. lini* completes all of its life stages on its flax host, transmission occurs across a wide range of environmental conditions, and infection is easily observed in situ.

To observe how processes of disease spread play out in different climate conditions, we studied four populations of *L. lewisii* spanning an elevation gradient of roughly 1,000 meters (Fig. 1B-E, Extended Data Fig. 1). At each site we monitored temperature, humidity, and rainfall (Fig. 1F-J). The large variation in these weather metrics between sites and across time allows for environmental drivers of transmission processes to be quantified via statistical modeling. We begin by analyzing the effects of climate change on the within-host processes of fungal replication, infection intensity progression, and plant growth. Next, we consider climate effects on transmission, linking spore deposition and environmental conditions to infection risk. Finally, we present a spatiotemporal epidemiological model, validate it against observed epidemiological patterns, and use it to project climate effects on population level patterns of disease spread.

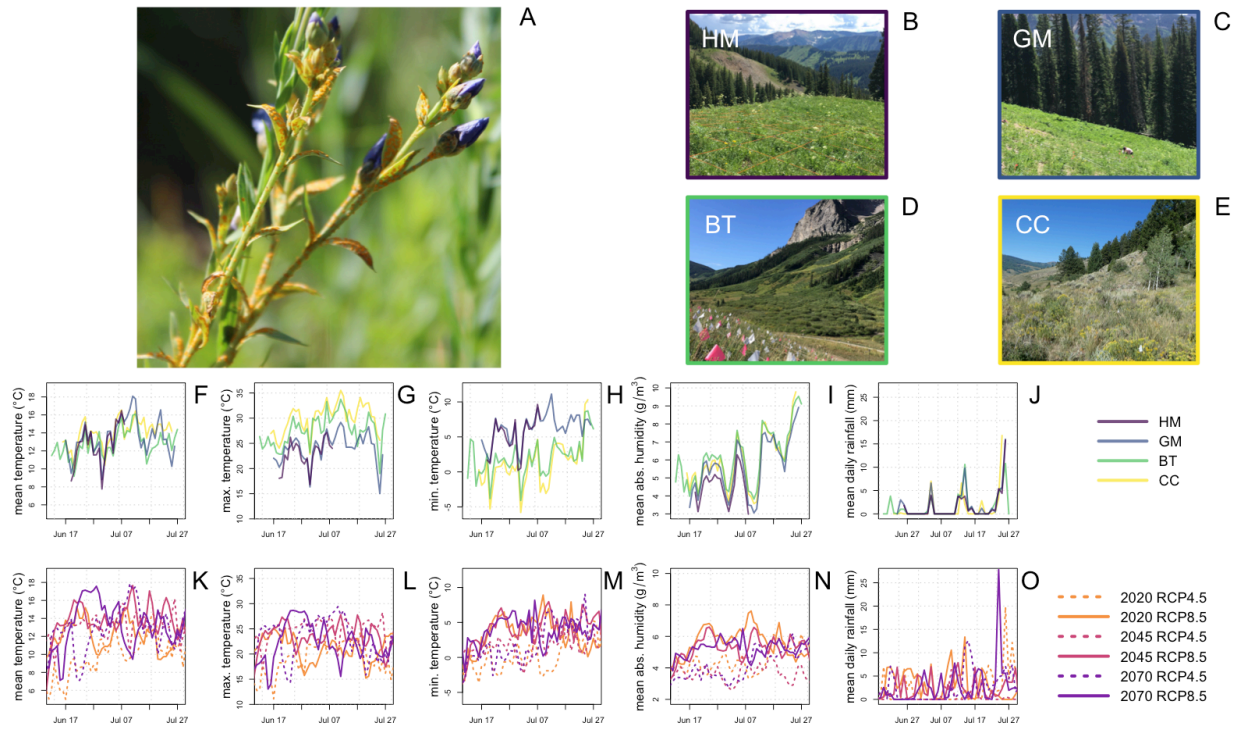


Figure 1: The flax rust study system

Panel A shows a *L. lewisii* plant infected with *M. lini*. Panels B-E shows photographs of the ‘high meadow’, ‘gothic mountain’, ‘bus turnaround’ and ‘cement creek’ (hereafter HM, GM, BT, CC) sites. These sites are located at approximately 2,440m, 2,940m, 3,200m, and 3,410m elevation respectively. Panels F-J show observed weather data collected at each site over the duration of the study period, which occurred in June and July of 2020. Panels K-O show weather data projected for the area surrounding the BT study site. These projections were generated for future climate scenarios encompassing the RCP 4.5 (dashed lines) and 8.5 (solid lines) emissions pathways for the years 2020 (year of observations, orange lines), 2030, 2040, 2045 (magenta lines), 2050, 2060, and 2070 (purple lines). The RCP 4.5 projections correspond to an intermediate scenario in which emissions begin to decline by approximately 2050 , and the RCP 8.5 projections correspond to a more pessimistic scenario in which emissions increase through 2100 [17].

Within-host processes

Replication rate

Replication rate is a key process underlying the spread of *M. lini*, as it produces the urediniospores necessary for disease proliferation. Understanding the effects of weather conditions on this trait is important for determining how and why changes in infection intensity progression might occur in future climate scenarios. We tracked longitudinal changes in pustule area (Fig. 2A, Fig. S1) as a proxy for fungal replication. To infer the effects of environmental conditions on pustule growth, we fit a generalized additive model [18] (GAM) of change in pustule area per day to this data, including smooth predictor terms for five climate variables: mean, maximum and minimum temperature; mean absolute humidity; and mean daily rainfall. These variables were selected because they have been previously found to affect either pustule growth or disease progression [13,19]. We also included random effects for study site and the identities of the ‘source plants’ on which the pustules were observed.

The fitted GAM points to the presence of an allometric effect of pustule size, with an increase in size expected for small pustules, and a decrease in size expected when pustule area exceeds approximately 0.2 mm² (Fig. 2B). Cooler mean temperatures and warmer minimum temperatures produced the largest positive effect on change in pustule area (Fig. 2C-E). Absolute humidity values less than ~6.5 g/m³ promoted pustule growth, while more humid conditions impeded pustule growth (Fig. 2F). A small amount of rainfall (up to ~0.5mm per day) promoted pustule growth (Fig. 2G), but the model indicated uncertainty about the effects of increased rainfall for greater amounts. In most cases, the identity of the source plant had a minimal effect on pustule growth. These fitted effects had a linear relationship with theoretical gaussian quantiles, indicating that they are approximately normally distributed (Fig. 2H). To infer how

climate change might affect patterns of *M. lini* replication, we simulated pustule area trajectories from the fitted GAM using weather data projected for future climate scenarios (see Fig. 1K-O). We used projected climate data rather than observed weather data to conduct simulations for 2020 to avoid biases introduced by data source in comparisons of results across years. The simulated growth trajectories indicate that in the RCP 4.5 emissions scenario, *M. lini* replication rate will gradually slow over time as climate change progresses. Replication rates were projected to remain relatively constant over time in the RCP 8.5 scenario, with a very slight positive trend from 2040-2070 (Fig. 2J, Extended Data Fig. 2).

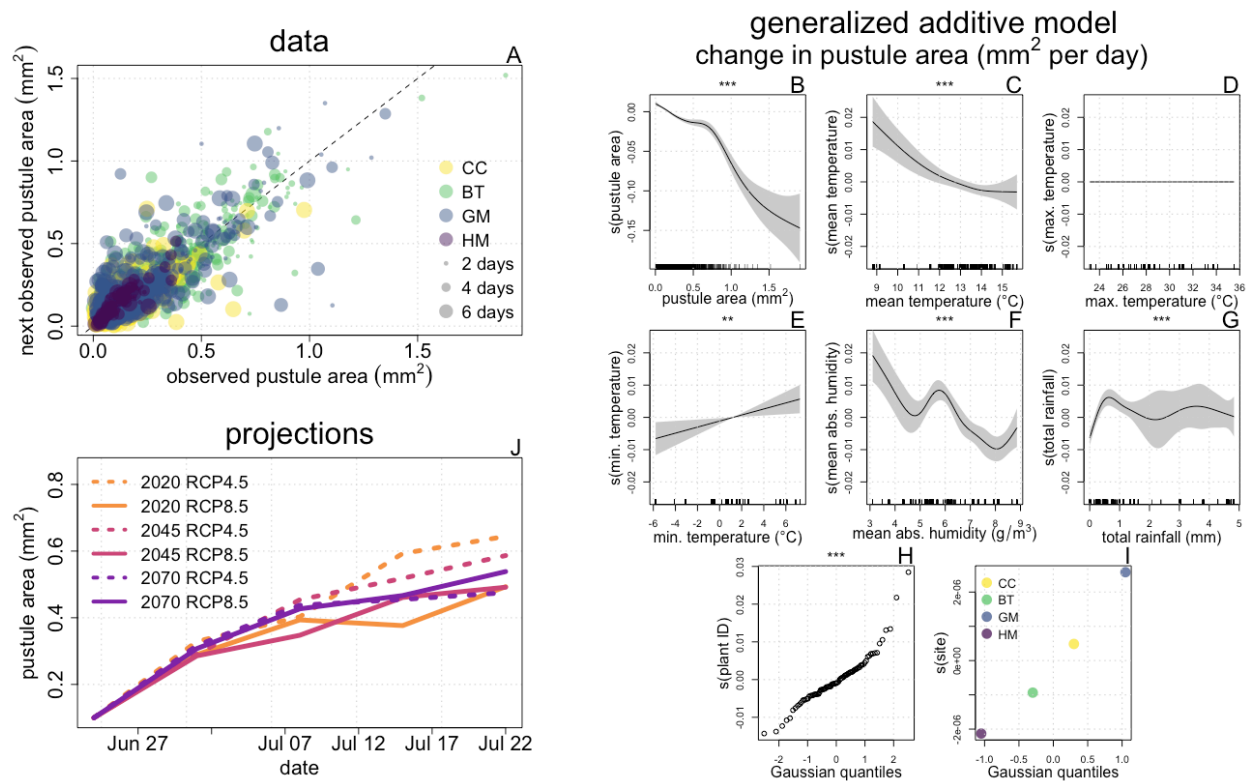


Figure 2: The effects of climate change on *M. lini* replication rate

Data: We made 3,319 observations of pustule area from 1,434 individual pustules located on 84 diseased focal plants. Panel A shows this data, with each point representing one observation. Color indicates study site, and size represents the elapsed time between observations. While changes in pustule area capture

pustule growth, they also capture processes that decrease pustule size (potentially including spore dispersal), so both positive and negative changes are expected. The dashed line shows $y=x$ to illustrate where increases (points above the line) and decreases (points below the line) in pustule area occur.

Generalized additive model: We fit a GAM with an identity link function to this data, using change in pustule area per day as the response variable and smooth terms for weather variables and pustule area as predictors. We also included source plant identity and study site as random effect smooths to account for the nested nature of our data. Panels B-I show the value of the fitted smooths (i.e. 's(x)') for different values of the predictor variables. Symbols above plots show the significance of smooth terms, with *** indicating $p < 0.001$, ** indicating $p < 0.01$, and * indicating $p < 0.05$. In the model fitting and selection procedure, smooth terms lacking explanatory power are set to have no effect and thus are functionally removed from the model, as seen in panel D. In panels B-E, lines show the fitted value of the smooth, and shading represents ± 2 standard errors. Ticks along the horizontal axis represent observed weather metrics. In Panels H and I, points show the fitted values of random effects vs. the quantile of their fitted value. *Projections:* Using the fitted GAM model, we 100 simulated trajectories of pustule growth for a small pustule (starting with area 0.1 mm^2) at the four study sites for each of six future weather data sets. Panel J shows the means of trajectories simulated for the GM site. These qualitatively match results for other sites (Extended Data Fig. 3).

Infection intensity progression

Next, we turn to the effects of climate on the progression of infection intensity. We tracked infection intensity in diseased focal plants (Fig. 3A, Fig. S2), calculating a unitless infection intensity score (designed to synthesize available measures to quantitatively capture the overall severity of infection) as the product of 1) the number of infected stems, 2) the average length of infected tissue per stem, and 3) the average number of pustules per leaf. Using this data, we again fit a GAM to infer how environmental conditions affect the rate of infection intensity progression. The fitted GAM (Fig. 3B-I) of change in infection intensity per day indicates that minimum temperatures less than 2°C have a positive effect on infection intensity progression (i.e. they increase infection intensity), while higher minimum temperatures have a negative effect (i.e. they decreased infection intensity, Fig. 3E). Increasing rainfall had a small positive effect

(Fig. 3I). The tensor product (Fig. 3B) revealed a complex interaction between infection intensity and height. All combinations of plant height and $\log_{10}$ infection intensity had a positive effect on infection intensity progression when $\log_{10}$ infection intensity was low (i.e. less than ~ 2.5), perhaps due to the high availability of plant tissue for the fungus to colonize. For heavily infected plants ($\log_{10}$ infection intensity between ~ 2.5 and ~ 4), different combinations of plant height and infection had either strong positive or weak negative effects, reflecting the diversity of observed infection intensity trajectories (Fig. S2). For very heavily infected plants ($\log_{10}$ infection intensity greater than ~ 4), most combinations of infection intensity and plant height had strong negative effects. This is potentially due to the rate of pustule degradation exceeding the rate of new tissue colonization when most tissue has been infected. While in most cases plant identity had a weak effect on infection intensity progression, the non-linear relationship between fitted effects and theoretical gaussian quantiles reflects a heavy-tailed distribution, indicating that infection may progress much faster or slower in some plants than in others (Fig. 3H).

We generated projections about how climate change might affect the progression of infection intensity by simulating infection intensity trajectories using the fitted GAM and future weather data projected for different climate scenarios. We found that climate change will initially slow the rate of infection intensity progression. Following this, a rebound in infection intensity progression will occur, although infection intensity levels are not projected to surpass the 2020 benchmark. Peak infection intensity was lower for RCP 8.5 scenarios than for RCP 4.5 scenarios (Fig. 3J, Extended Data Fig. 4).

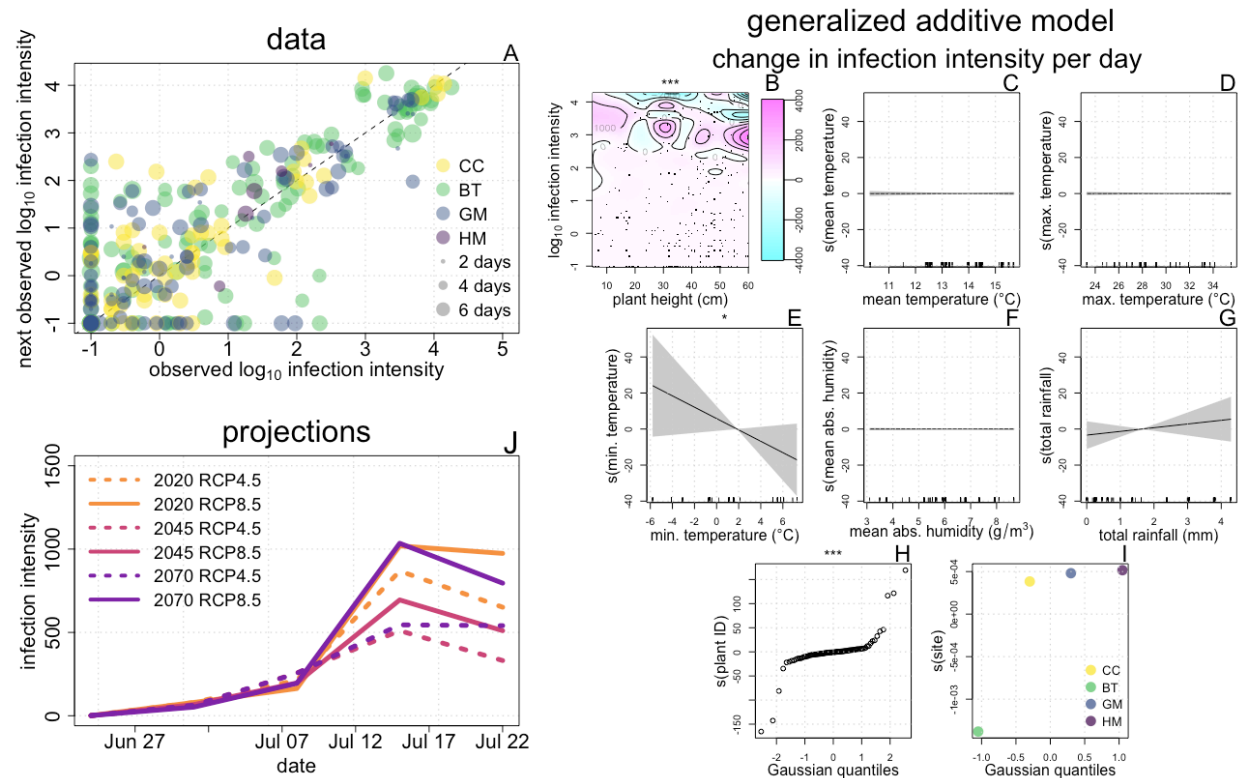


Figure 3: The effects of climate change on the progression of *M. lini* infection intensity

Data: Across 90 diseased focal plants, we made 313 observations of infection intensity (Panel A). Both increases and decreases in infection intensity are expected, as both the proliferation of infection and the deterioration of infected tissue may occur. Panel A shows $\log_{10}$ transformed observed data, with each point representing one observation. Point color indicates study site, and point size represents the elapsed time between observations. The dashed line indicates $y=x$ to illustrate where increases and decreases in infection intensity occur. *Generalized additive model:* We fit a GAM of change in infection intensity per day, using an identity link function. Predictors included smooth terms for environmental conditions, a full tensor smooth product (which captures both individual and joint effects of predictors) of $\log_{10}$ infection intensity and plant height, and random effect smooths for plant identity and site. We expect height and infection intensity to have a joint effect for two reasons. First, height can partially constrain infection intensity by limiting the amount of tissue that can be infected. Second, quantitative resistance, which could slow the spread of infection within a plant, could be correlated with plant size. Panel B shows the fitted tensor product, with colors and contours indicating the tensor value (analogous to smooth value) for different combinations of plant height and $\log_{10}$ infection intensity. Points indicate observed data. Panels C-I show the fitted smooth terms, with axes, lines, and shading as in Fig. 2B-G. Panels H and I show

fitted random effects, with points and axes as in Fig. 2H-I. Symbols above plots show the significance of smooth terms, with *** indicating $p < 0.001$, ** indicating $p < 0.01$, and * indicating $p < 0.05$. *Projections:* We simulated 100 trajectories of infection intensity for a lightly infected plant (infection intensity 1) of approximately average height (25 cm) at each site for six future weather data sets using the fitted GAM. The means of these trajectories, simulated for the GM site, are shown in panel J. These results qualitatively match those simulated for other sites (Extended Data Fig. 5).

Plant growth

Impacts of climate variables on fungal growth alone do not capture the range of impacts of fungal populations. The effects of climate on plant growth are also relevant due to the role plant height plays in i) regulating the progression of infection intensity (Fig. 3B), ii) determining patterns of spore dispersal, and iii) modulating the risk of infection (see Transmission section below for background on ii and iii). Using plant height data collected from infected and uninfected focal plants (Fig. 4A, Fig. S3), we fit a GAM to characterize how environmental conditions and infection intensity affect plant growth. The fitted model (Fig. 4B-I) indicates that the rate of plant growth declines as maximum daily temperature and absolute humidity increase, and that there is no significant stunting effect of infection.

To infer how climate change might affect patterns of plant growth in *L. lewisii*, we simulated growth trajectories using the fitted GAM and future weather data projected for future climate scenarios. Broadly, growth rates are projected to be higher in the RCP 4.5 scenario compared to the RCP 8.5 scenario. Climate change will slow the rate of plant growth over time in the RCP 4.5 scenario, but in the RCP 8.5 scenario, growth rates are expected to remain approximately constant except for a transient increase in 2030. (Fig. 4J, Extended Data Fig. 6).

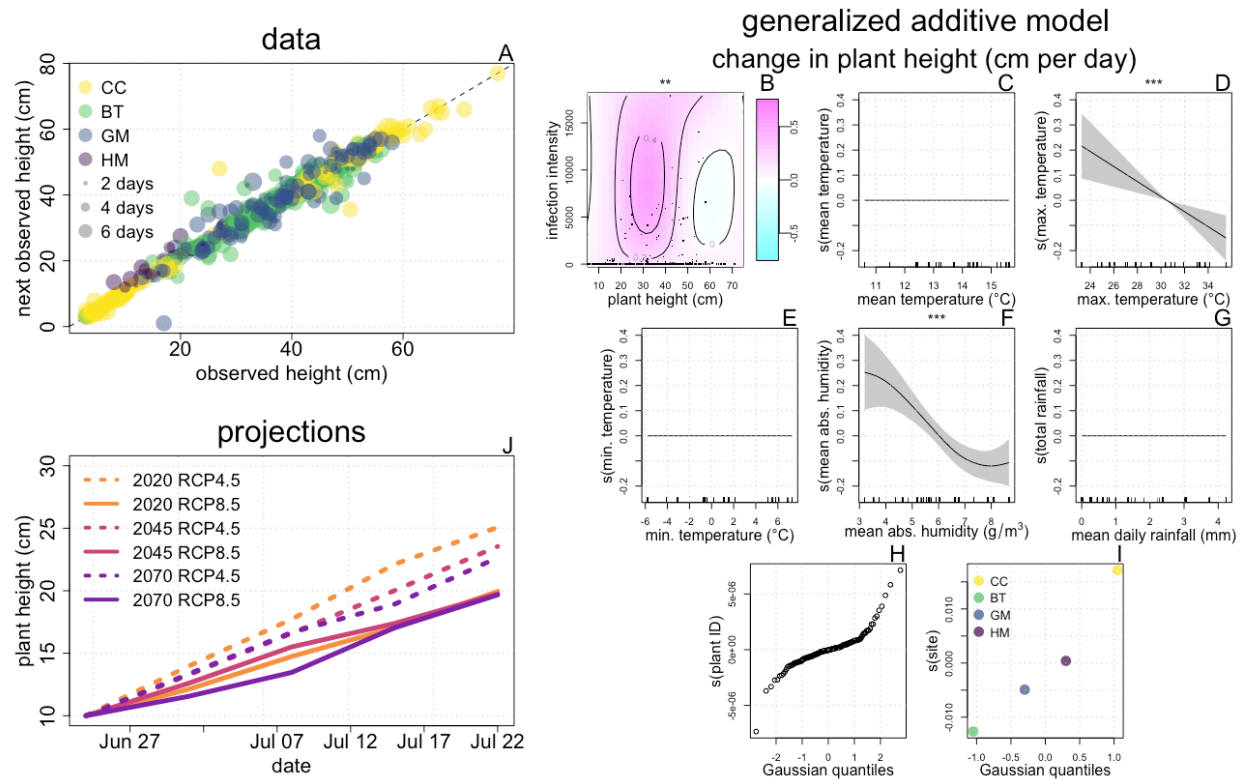


Figure 4: The joint effects of climate change and infection on *L. lewisii* growth

Data: We made a total of 596 observations of plant height from 175 focal plants (panel A). Points show individual observations with color indicating site and size indicating elapsed time. Unsurprisingly, we observed both increases and decreases in plant height as plant condition changed over the course of the observation period. The dashed line illustrates $y=x$ to show where increases and decreases occurred.

Generalized additive model: We fit a GAM to this data, using change in plant height per day as the response variable, and smooth terms for weather variables as predictors along with a full tensor product of height and infection intensity. Plant identity and site were included as random effect smooths. Panel B shows the fitted tensor, with contours, shading, and points as in Fig. 3B. Panels C-G show fitted smooth terms with axes, lines, and shading as in Fig. 2B-G. Panels H and I show fitted random effects with points and axes as in Fig. 2H-I. Symbols above plots show the significance of smooth terms, with *** indicating $p < 0.001$, ** indicating $p < 0.01$, and * indicating $p < 0.05$.

Projections: We simulated 100 growth trajectories for a small plant (starting with a height of 10 cm) at each site for six future weather data sets using the fitted GAM. The lines in panel J show means of the trajectories simulated for the GM site.

Qualitatively similar patterns were observed in simulations performed for other sites (Extended Data Fig. 7).

Transmission

After inferring the effects of environmental conditions and climate change on within-host processes, we set about connecting the within-host spread of disease to between-host patterns of transmission. First, we collected data on the spread of *M. lini* within *L. lewisii* populations, tracking the spread of infection through space and time at each of the four study sites. We began by mapping the location of each plant in a 10 by 20 meter transect at each study site. We then inspected each plant for the presence of infection approximately every seven days.

Next, we sought to connect infection intensity and plant height to spore dispersal. We deployed spore traps in arrays around diseased plants, and counted the number of *M. lini* spores caught in each trap. Using this data, observed patterns of wind speed and direction, and measurements of source plant infection intensity and height, we parameterized a tilted gaussian plume model [20] (TGPM) describing spatial patterns of spore dispersal (see methods, Figs. S4-6). As the TGPM predicts the number of spores per mm² deposited at the coordinates of a target plant, rather than the number of spores coming into contact with the plant, we present spore dispersal as a unitless metric that captures the relative intensity of spore deposition experienced by a plant.

Finally, we inferred the relationships between environmental conditions, spore deposition, and infection risk. We used the TGPM to predict the spore deposition experienced by each healthy plant in each study population between observations of the infection status (Fig. 5A). We then modeled the relationship between log₁₀ predicted spore deposition, environmental conditions, and infection occurrence using a GAM (Fig. 5 B-I). The fitted GAM indicated the presence of a thermal optimum for transmission, with the odds of transmission increasing with warmer minimum temperatures (Fig. 5C), but decreasing with warmer mean temperatures (Fig.

5E). Unsurprisingly, the odds of transmission rose with increasing spore deposition (Fig. 5J). For a given level of $\log_{10}$ predicted spore deposition, infection odds generally increased with plant height (Fig. 5B, Extended Data Fig. 9). This is intuitive, because the TGPM predicts spore deposition for a point in space, but larger plants represent bigger ‘targets’ for spore deposition, and should experience greater actual spore deposition for a given level of predicted spore deposition. Extended Data Fig. 9 illustrates the details of the relationships between plant height, spore deposition, and time inferred by the fitted GAM.

We investigated how climate change might affect infection risk by simulating the fitted GAM using weather data projected for future climate scenarios. We observed that climate change is expected to initially lower infection risk, but that a resurgence in risk is expected as climate change continues. The resurgence in risk projected for 2070 exceeds the 2020 baseline in the RCP 8.5 scenario but not in the RCP 4.5 scenario. These results indicate that in the short term, climate change might lower infection risk, but this reduction will be transient if emissions continue to increase over time (Fig. 5J, Extended Data Fig. 8).

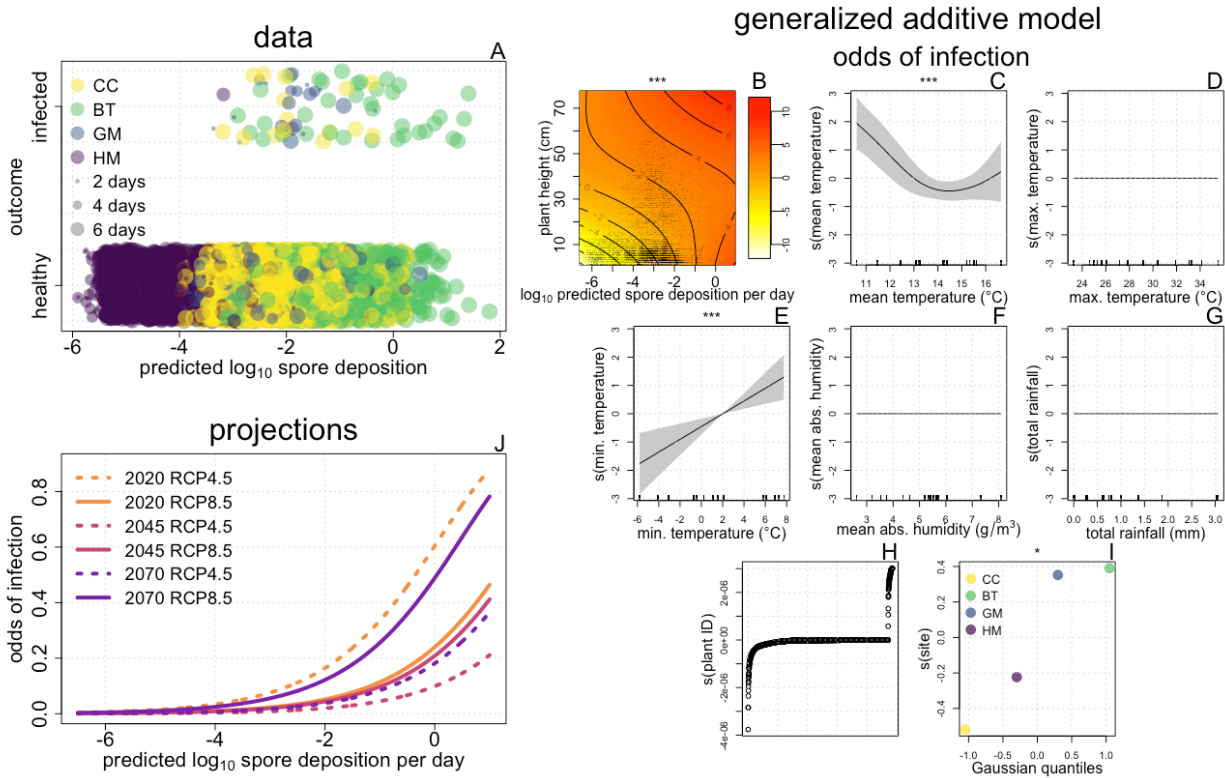


Figure 5: The effects of climate and spore deposition on infection risk

Data: We predicted the total spore deposition experience by each uninfected plant at each study site over the periods between observations of infection status. We made these 9,142 predictions using the TGPM in conjunction with observed wind speed, the locations of infected plants, and the infection intensities and heights of those plants. Points in panel A show infection outcome vs $\log_{10}$ predicted spore deposition, with the color and size of the points representing study site and the time between observations.

Generalized additive model: We fit a GAM to this data using infection outcome as the response variable and assuming a binomial response distribution. We used a complementary log-log link function and included an offset of log time to account for variation in the length of the observation period. Predictors included smooth terms for weather variables, random effect smooths for plant identity and site, and a full tensor product of $\log_{10}$ predicted spore deposition per day and plant height. Panel B shows the fitted tensor, with contours, shading, and points as in Fig. 3B. Panels C-G show fitted smooth terms with axes, lines, and shading as in Fig. 2B-G. Panel H shows fitted random effects for plant identity plotted in the order of their magnitude. Panel I shows fitted random effects for site with points and axes as in Fig. 2I. Symbols above plots show the significance of smooth terms, with *** indicating $p < 0.001$, ** indicating $p < 0.01$, and * indicating $p < 0.05$. *Projections:* Lines in panel J show the relationship between $\log_{10}$

predicted spore deposition per day and the odds of infection (for a plant with a height of 25cm at the GM site) projected from the fitted GAM for the time period spanning July 7th-July 14th. We replicated these predictions across weather data sets projected for six climate scenarios.

Epidemiological model

In the final step of our analysis, we extended our inferences about the effects of environmental conditions on within-host processes and transmission to generate predictions about the effects of climate change on the spread of infection at the population scale. To accomplish this, we constructed a spatiotemporal epidemiological model using the parameterized TGPM and fitted GAMs of infection odds and within-host processes. We validated the model by simulating epidemics at the BT and GM sites using observed weather data and confirming that predictions qualitatively recapitulated observed epidemiological patterns (Fig. 6A,B). Epidemics simulated from observed weather data at the GM site slightly underpredict prevalence by the end of the observation period (Fig. 6B). A potential explanation for this slight discrepancy could be that infected plants observed just outside of the transect may have contributed to spore deposition in the transect. To infer the effects of climate on population level disease spread, we again simulated epidemics at the BT and GM sites, this time using weather data sets projected for future climate scenarios. These results represent projections of how the observed epidemic might have progressed if it had played out in future climate conditions.

These results of these simulations (Fig. 6C,D, Extended Data Fig. 10) reveal that climate change is likely to slow the spread of disease in the short term, but that continued climate change will likely lead to a resurgence in disease spread, albeit one that falls short of current levels. This pattern was observed for both the RCP 4.5 and 8.5 emissions scenarios. Our simulations predict greater reductions in the pace of disease spread in the short term for the RCP 8.5 scenario, as

prevalence in 2020-2040 was projected to be higher in this emissions pathway as compared to the RCP 4.5 pathway. However, increased emissions are projected to amplify the resurgence in disease spread, as projections for the RCP 8.5 scenario match or exceed those for the RCP 4.5 scenario by 2070.

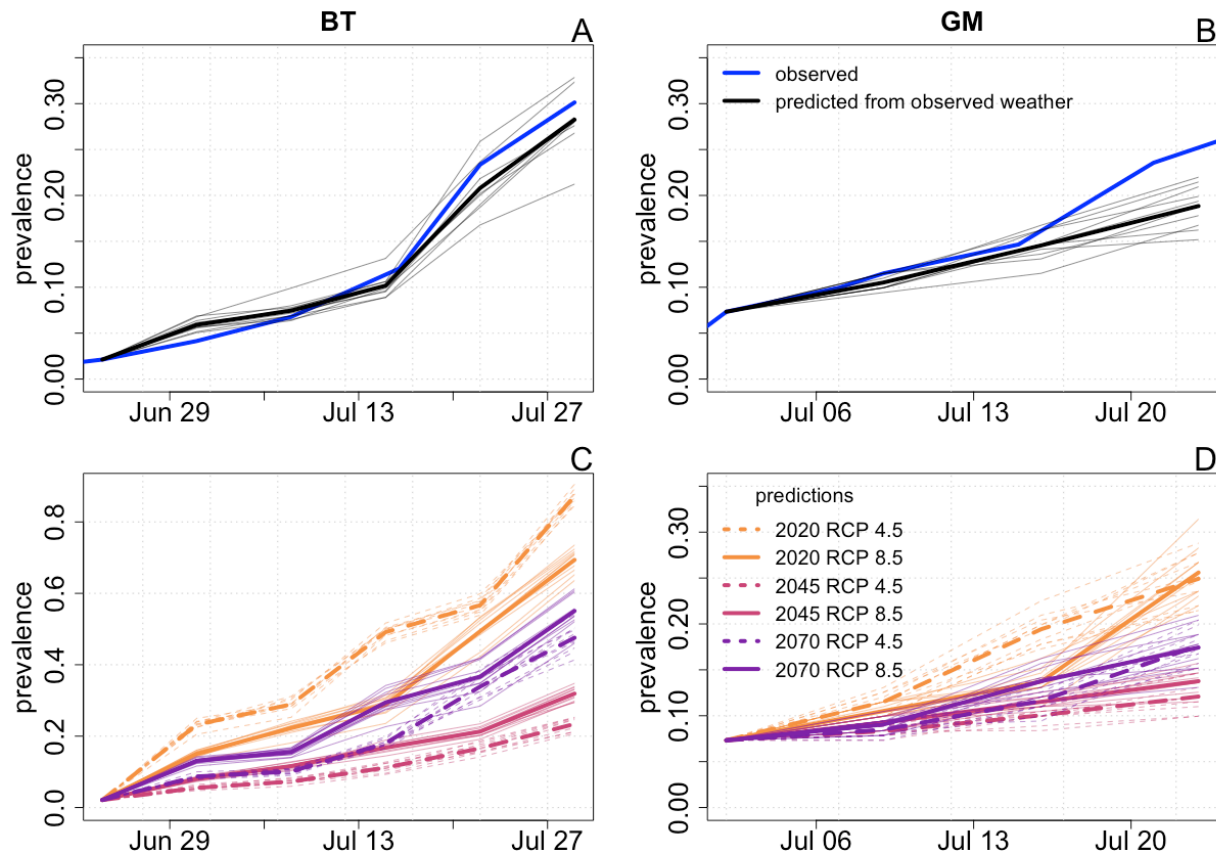


Figure 6: The effects of climate change on the epidemiology of flax rust disease

Panels A and B show the observed patterns of infection prevalence at the BT and GM sites (blue lines) along with prevalence simulated from 10 runs of the spatiotemporal epidemiological model using observed weather data (thin black lines). The thick black lines show the mean of the 10 simulations. Panels C and D show patterns of infection prevalence at the BT and GM sites simulated for six future climate scenarios. We simulated 10 epidemics at each site for each climate scenario. Thin lines show prevalence predicted from individual simulations, and thick lines show mean prevalence across 10 simulations for a given climate scenario.

Discussion

Climate change is predicted to drive changes in the spread of disease in the flax rust plant pathosystem across multiple scales of transmission. At the within-host scale, plant growth will slow, and infection will spread less rapidly, perhaps due to a decrease in the pathogen replication rate associated with rising mean temperatures. This will lead to a drop in infection risk due to both a reduction in spore dispersal and a decrease in the odds that a plant becomes infected when challenged with fungal spores. This combination of effects will decelerate the speed of epidemics at the population level. However, this slowing of transmission processes across scales is predicted to reverse as climate change continues, especially if greenhouse gas emissions increase (i.e. RCP 8.5 scenario). Rebounds in plant growth, infection intensity progression, infection risk, and epidemic pace are all predicted to occur after the initial deceleration.

A likely explanation for these predicted rebounds pertains to the details of the projected future weather data used to generate these predictions. June to early-July temperatures are warmer in the projected 2070 weather data compared to the projected 2045 data, but the opposite is true for mid-to-late July temperatures (Fig. 1K-M). Given the negative effect of increased temperature on disease spread at multiple scales, this difference in weather patterns is a probable explanation for disease resurgence. Broadly, this points to the importance of considering both the magnitude and timing of differences between current and future climate.

By using flax rust as a model for fungal plant pathogens in general, we can identify several important impacts of climate change on the dynamics of these diseases. While flax rust may not be a universally representative fungal pathogen, especially for systems with different thermal ecologies, our results can serve a much needed first approximation in most cases. In agricultural systems, there may be unexpected upsides associated with warming in the short

term, as infection intensity and prevalence may decrease. However, we found that climate change is expected to stunt flax growth. If these patterns hold in crops, then the net effect of climate change will depend on the balance between increases in yield due to disease reduction and decreases in yield due to slower growth. In natural populations, the favorability of outcomes associated with climate change will be system-specific. Some species of conservation importance may benefit, such as Australian myrtle species (genus *Myrtaceae*) threatened by the rust fungus *Austropuccinia psidii* [21], as increases in temperature slow disease development and epidemic spread [22]. In other systems, the threats posed by invasive species held in check by disease [23,24] may increase. Ecosystem-level effects are likely to be complex, especially if multiple interacting pathogen and host species are affected.

In this study, we focused on the effects of climate change on patterns of transmission within a single epidemic. However, several factors not accounted for in our epidemiological model may also have significant direct and indirect effects on the relationship between climate and impacts of disease. The effects of climate change on disease phenology (the duration and timing of the period during which environmental conditions render host condition and pathogen physiology adequate for disease spread [25]) could boost end-of-epidemic prevalence by lengthening the duration of conditions suitable for flax rust spread. Likewise, demographic feedbacks are important to consider [26]. Indeed, disease and climate effects on host mortality and demography have been linked to the presence of multi-annual epidemic cycles in the *Linum marginale*-*M. lini* flax rust system [13]. Analogous patterns exist in human systems such as measles, in which birth and vaccination rates, which in combination determine the rate of susceptible host recruitment, are linked to the periodicity of outbreaks [27]. Feedbacks between climate and evolutionary processes will also affect the dynamics of plant disease spread. Both

‘quantitative’ and ‘qualitative’ pathogen virulence and host resistance traits are important to consider in this context. ‘Qualitative’ traits jointly determine whether infection occurs, while ‘quantitative’ traits dictate the extent to which the pathogen exploits the host [28]. The *L. marginale*-*M. lini* flax rust system has been studied extensively as a model for the co-evolution of qualitative traits [29,30], and associated epidemiological outcomes [31]. The effects of climate on replication rate may have consequences for selection on qualitative virulence, as these two traits are known to be linked [30]. Quantitative traits have also been studied in flax rust [32], but have not been connected to epidemiological patterns. Our finding that warmer temperatures may slow infection intensity progression suggests that climate change might select for increased quantitative virulence in *M. lini* because a more aggressive host exploitation strategy could help the pathogen recoup transmission losses.

In this study, we generated quantitative predictions about how plant growth, disease severity, and infection prevalence in the flax rust system might change in future climates. These results can serve as a first approximation for how the dynamics of other fungal diseases of plants may change. More broadly, this effort represents an important bridge between the fields of infectious disease dynamics and plant pathology by introducing a modeling framework for making semi-mechanistic projections of plant infectious disease spread in future climates. Developing a more complete understanding of plant disease in a changing world will require further integrations of approaches and ideas from each field.

Methods

Data collection

Study sites

We selected four study sites in the Upper Gunnison Basin in Colorado, USA. As we intended for these sites to capture a large amount of variation in environmental conditions, the primary criteria we used for site selection was elevation. Mirroring patterns of mountain weather observed worldwide, elevation is correlated with a multitude of environmental factors in the Rocky Mountains. Predominant trends include a negative correlation between altitude and temperature, a positive correlation between altitude and precipitation [33], and transitions in plant community composition (from ‘mountain shrub’ to ‘montane’ to ‘subalpine’ to ‘alpine’) with increasing altitude [34].

Our sites span a large elevation gradient of approximately 1,000 meters that captures significant variation in environmental conditions. These sites should not be interpreted as a direct ‘elevation for climate substitution’, especially considering the significant changes in weather at each site that occur over the course of our observation periods, which ran from June to July. The lowest site, ‘cement creek’ (CC), falls within a sage brush meadow on the boundary between the mountain shrub and montane vegetation zones at 2,440 meters elevation. The second lowest site, ‘bus turnaround’ (BT), is situated in an open subalpine meadow at the base of Gothic Mountain at ~2,940 meters. The second highest site, ‘gothic mountain’ (GM), is also in the subalpine zone on a steep hillside within a clearing in an evergreen forest on the lower slopes of Gothic Mountain at 3,220 meters. The highest site, ‘high meadow’ (HM), is on an exposed meadow at the upper fringes of the subalpine zone on the shoulder of Gothic Mountain at 3,410 meters.

We began our observations at each site by mapping the flax population (see *Population mapping* sub-section below). This occurred on 6/15/2020 for CC, 6/17/2020 for BT, 6/16/2020 for GM, and 6/18/2020 for HM. We concluded all observations at CC on 7/27/2020, at BT on 7/29/2020, at GM on 7/28/2020, and at HM on 7/10/2020 (aside from spore trap collection which concluded on 7/21/2020).

Weather monitoring

We deployed environmental sensors to record longitudinal weather data at each of our sites. We recorded temperature, humidity, rainfall, and wind speed and direction (at ~1 meter above ground) every five minutes (see Supplementary Text 1 for logger specifications). We used temperature and humidity data to calculate absolute humidity (Supplementary Text 2). Due to logistical constraints, rainfall and wind speed/direction loggers were deployed later than temperature and humidity loggers.

We extracted weather metrics for a given observation period from this data as follows: We calculated the mean, maximum, and minimum temperature as the mean, maximum, or minimum of temperature readings. Likewise, we calculated mean absolute humidity as the mean of absolute humidity records (calculated from temperature and relative humidity every five minutes). Daily mean rainfall was calculated as of mean rainfall records multiplied by the number of 5 minute increments in a day (i.e. 288).

Future climate data

To enable forecasting of the effects of climate change on within and between host transmission processes, we compiled projected future weather data. We extracted downscaled climate and

hydrological data generated using the Localized Constructed Analogs (LOCA) method and the CESM1(CAM5) model from the “Downscaled CMIP3 and CMIP5 Climate and Hydrology Projections” archive [35–39]. Specifically, we extracted values for daily mean temperature, relative humidity, and rainfall from the CESM1(CAM5) hydrology model, and values for daily maximum and minimum temperature from the CESM1(CAM5) climate model for both the RCP4.5 and RCP8.5 emissions scenarios [40]. Temporally, this data spanned June through July in 2020, 2030, 2040, 2045, 2050, 2060, and 2070. Spatially, this data represents projections for the area spanning (38.9375, 39.0) latitude and (-107.0, -106.9375) longitude. This area is the finest grained spatial extent including the BT site for which projections were available.

Population mapping

In order to uncover drivers of population level epidemiological dynamics, it is necessary to characterize the ‘landscape of susceptibility’ in the host population. For spatially structured infectious disease systems such as flax rust, this involves documenting not only the infection status of each individual and any likely covariates of contact rate or susceptibility (e.g. size), but also the spatial arrangement of individuals. To accomplish this, we established 10 by 20 meter transects at each of our field sites. We recorded the coordinates of each transect corner and the compass bearings associated with the transect axes (so that wind direction could be translated to the transect coordinate system) using a Garmin GPS unit (part #: 010-01735-10). At the beginning of each field season, we mapped the location, height, and infection status (healthy or diseased) of each plant within each transect. Plants with heights less than five centimeters were denoted as seedlings. While other metrics of size such as stem count could be used to form a more complete picture of plant size, we chose to use height alone as a proxy for size as it was the

only measure that we could feasibly record for the many hundreds of plants within each transect. At the CC site, we were unable to map all healthy plants in certain areas of the transect, but we did record data for all diseased plants in these regions.

Epidemiological surveys

After documenting the initial epidemiological conditions, we tracked the spatiotemporal spread of flax rust within each transect. Approximately once per week, we inspected each flax individual in the transect for signs of disease, recording the location of any newly infected plants so they could be matched to a previously uninfected plant. We also recorded the height of all newly infected plants. Plants identified as infected in the initial population mapping and in these subsequent epidemiological surveys were marked with flags to ensure that they would not be repeatedly recorded as newly infected. Although we did not map the entirety of the CC transect, we covered the entire 10 by 20m area in all epidemiological surveys.

Diseased focal plants

Within each transect, we designated a subset of diseased plants as ‘focal diseased plants’ and used them to make detailed measurements of within-host disease spread processes. These processes included changes in infection intensity, which we measured at the plant scale, as well as pustule growth, which we measured at the sub-plant scale using changes in pustule area and as a proxy.

All initially infected plants were designated as diseased focal plants, and we continued to give this designation to newly diseased plants (except in some cases, diseased plants with height of approximately 5cm or less, as these plants were too delicate to be manipulated) until we

obtained at least 25 infected focal diseased plants per site. This occurred on 7/20/2020 at CC, on 7/1/2020 at BT, and on 7/9/2020 at GM and HM. We inserted metal tags into the ground at the base of diseased focal plants so they could be quickly and reliably identified. To facilitate longitudinal measurements of disease spread at sub-plant scales, we marked up to three infected stems on each focal diseased plant with pieces of colored flagging tape tied around the base of the stem. These stems were chosen haphazardly. On each of these stems (when possible), we marked the tip of one infected leaf with black ink. We preferentially marked leaves with one or a few pustules. Approximately once every three days, we recorded detailed measurements of plant height and within-host disease spread processes.

To measure pustule growth, we photographed each marked leaf with a millimeter ruler in frame using a Cannon EOS Rebel T7i DSLR camera fitted with a Canon EF-S 35mm f/2.8 Macro IS STM lens and a Cannon MR-14EX II Macro Ring Lite. While achieving consistent photographic angles and lighting conditions is impossible in a field setting, we attempted to keep the leaf and the scale in the same plane while maintaining a perpendicular shooting angle. We also adjusted camera settings so that pustule boundaries could be clearly distinguished in images. We used ImageJ software to measure the maximum and minimum diameters of each photographed pustule, and calculated pustule area assuming pustules to be elliptical. To enable the size of individual pustules to be tracked across observations, each measured pustule in each image was labeled so that it could be re-identified in subsequent images based on its location on the leaf and position relative to other pustules. We omitted data for any pustule that could not be confidently identified or whose borders became indistinct from other pustules. We marked a new leaf when we were unable to find the previously marked leaf, when the leaf was accidentally removed during data collection, or when the condition of the leaf deteriorated significantly. We

suspect that either the presence of ink or our handling of the plant contributed significantly to the rate at which leaf condition deteriorated, but nevertheless we were able to observe the growth of many pustules over many weeks.

To measure infection intensity, we first counted the number of infected stems. Next, for each marked stem, we recorded the length of infected tissue as the length of the stem segment spanning the lowest (relative to the ground) pustule to the highest pustule. Finally, we counted the number of pustules present on a haphazardly selected leaf in the middle of the region of infected tissue on each marked stem. We did not use the same leaf at each observation as the middle of the region of infected tissue changed due to disease spread. Using these measurements, we calculated an infection intensity metric as the product of the number of infected stems, the average length of infected tissue, and the average number of pustules present on a leaf in the middle of the region of infected tissue.

Healthy focal plants

As we wished to investigate patterns of growth in both healthy and infected plants, we designated certain uninfected plants as healthy focal plants, and measured their height whenever we measured the height of focal diseased plants. To control for potential effects of stem marking on the growth of diseased focal plants, we made these same markings on healthy focal plants. Healthy focal plants were selected haphazardly, and we added new healthy focal plants over time to keep the number of diseased and healthy focal plants approximately equal. At some sites, the number of healthy focal plants fell towards the end of the observation period as many healthy focal plants became infected. When this occurred, we stopped recording data for the plant, and in some cases designated it as a diseased focal plant.

Spore deposition

To measure the distribution of spore deposition from an infected plant, and how it relates to the infection intensity of the source plant and wind patterns, we deployed spore traps in arrays around a subset of focal diseased plants. We chose focal disease plants that were as removed as possible from other diseased plants to minimize the number of spores originating from other plants that would be caught in the spore traps. These spore traps consisted of a $\sim 2 \text{ cm}^2$ section of Scotch Permanent Clear Mounting Tape (part #: MT76272-5) affixed to plastic backing with double sided tape. These spore traps were secured into the ground with $\sim 5 \text{ cm}$ nails. We deployed these traps at distances of either $\{\sim 5 \text{ cm}\}$ (traps at $\sim 5 \text{ cm}$ were placed as close to the plant as possible), $\{\sim 5 \text{ and } 25 \text{ cm}\}$, $\{\sim 5, 25, \text{ and } 50 \text{ cm}\}$, or $\{\sim 5, 25, 50, \text{ and } 100 \text{ cm}\}$ in each of the four directions corresponding to the axes of the transect in which they were located. The traps were left in the field for approximately one week, and then collected. The sections of mounting tape were transferred to microscope slides and sealed in place using clear packing tape. We then used a light microscope to count the number of spores present in a known area. We counted spores in an area of 0.8 cm^2 for most spore traps that contained few or no spores, but we counted a minimum area of 0.1 cm^2 for all spore traps, including those with greater than 10,000 spores per cm^2 . *M. lini* spores were identified visually using a slide prepared with spores as a reference for spore size, shape, and color. The spores matched descriptions in the literature⁴¹. We did not observe any rust fungi other than *M. lini* in or around our transects, so we can be reasonably certain that our counts represent only *M. lini* spores from infected focal diseased plants.

Statistical Analyses

Plant growth and within-host disease spread

To infer how different weather factors affect plant growth, pustule growth, and changes in infection intensity, we fit generalized additive models. We used longitudinal observations of plant height in healthy and diseased focal plants to fit the model of plant growth, and longitudinal observations of within-host disease spread at various scales in focal diseased plants to fit all other models. Because the observation periods associated with our measurements of these processes varied, we formatted the response variables in change-per-day units: For our analyses of plant growth, pustule growth, and infection intensity progression we used change in plant height per day, change in pustule area per day, and change in infection intensity per day respectively as the response variables.

To infer the effects of weather variables, we included smooth terms for these factors as predictors in each model. For each observation of change in plant height, pustule area, or infection intensity, we determined the start and end timepoints of the observation period to extract the corresponding weather data. For observations of plant height, we used 12:00:00 on the day of the observation as the start/end timepoints of the observation period. For observations of pustule area, we used photograph timestamps as the start and end timepoints. For observations of infection intensity, we used the average of the timestamps of photographs taken of pustules on that plant's leaves as the start and/or end timepoints if such photographs were taken, because all data for an individual diseased focal plant was generally collected at once. If such photographs were not taken, we used the mean timestamp of all photographs taken at that plant's site on the observation date as the start and/or end timepoint. To focus our analysis on fine-grained relationships between weather and within-host disease spread, we discarded data corresponding

to observation windows of eight or more days. Using the start and end timepoints of the observation period as bounds, we extracted the following variables from the weather data: temperature (mean, maximum, and minimum), absolute humidity, and mean daily rainfall.

To account for the nested nature of our data, we included smooth terms for study site and plant identity as random effects (accomplished by setting `bs="re"` in the `s()` function in `mgcv`) in each model. In the case of the analyses of pustule growth, plant identity refers to the plant on which the pustules were observed. We also included a term in each model that accounted for the value of the previous observation to capture any allometric effects. In the model of change in plant height per day, this term was a full tensor smooth product of last observed height and infection intensity. In the model of change in pustule area per day, this term was a smooth of last observed pustule area. In the model of change in infection intensity per day, this term was a full tensor smooth product of the base-10 logarithm of the last observed infection intensity and last observed maximum plant height.

We fit these models using the `gam()` function in the `mgcv` R package and the restricted maximum likelihood parameter estimation method [18,42]. We used gaussian response distributions with identity link functions. The dimensionality of all basis functions was set to the default value determined by `mgcv`. The one exception to this involved the marginal basis for $\log_{10}$ infection intensity (a part of the tensor term) in the model of change in infection intensity per day. The dimensionality of this marginal basis was increased to 15 after model diagnostics indicated that the default basis dimension was insufficient. To implement variable selection so that insignificant predictor terms would be effectively removed from the models, we used the double penalty approach of Mara and Wood [43] (via the `'select=TRUE'` option in the `mgcv` `gam()` function).

The coefficients of the fit models can be used to infer the effects of weather factors on plant growth and within host disease spread. To translate these inferences into predictions about how climate change might affect plant growth and within-host disease spread, we simulated trajectories of plant growth, pustule area, and infection intensity under various climate conditions. For each class of simulation, we started by defining a hypothetical starting state. For simulations of plant growth, this was a 10 cm tall uninfected plant (the plant was assumed to remain uninfected for the entire simulation). For simulations of pustule growth, this was a pustule with area 0.1 cm^2 . For simulations of infection intensity, this was a 25 cm tall plant with infection intensity 1.0. Next, we simulated 100 trajectories from the model posteriors using observed weather data for each site and a step size of seven days. In these simulations, we restricted plant height, pustule area, and infection intensity to remain greater or equal to 5 cm, 0 mm^2 , and 0.1 respectively. We included the random effect of site (but not plant identity) when making these predictions. After confirming that these simulation results qualitatively recapitulated observed patterns, we simulated sets of 100 trajectories for future climate data sets. We again excluded the random effect of plant identity when making these projections. We used the same set of 100 random number seeds for each set of 100 trajectory simulations.

While performing these simulations, we extracted weather variables as follows: When extracting weather variables from observed data sets, we defined the observation period as 00:00:00 on the first day in a step to 00:00:00 on the last day in a step (e.g. midnight on July 1st to midnight on July 8th). We extracted weather metrics as described in the Weather data subsection of the Data collection section above. Applying these same methods to the projected weather data is not possible, as these data sets only give only daily mean values. Instead, we calculated mean temperature as the mean of daily mean temperature values, maximum

temperature as the maximum of daily maximum temperature values, and minimum temperature as the minimum of daily minimum temperature values. Similarly, we used daily mean temperature and relative humidity to calculate daily mean absolute humidity, and calculated mean absolute humidity as the mean of these values. We applied this same procedure to calculate mean rainfall.

Spore dispersal

To infer how spore deposition is related to source plant infection intensity, wind speed, and wind direction, we fit a tilted gaussian plume model [20] (TGPM) to our spore deposition data. The equation specifying the TGPM is as follows:

$$d(I, H, s, X, Y) = \frac{I k W_s}{2 \pi s \sigma^2} e^{\frac{-Y^2}{2\sigma^2} - \frac{(H - \frac{W_s X}{s})^2}{2\sigma^2}} \text{ for } X > 0 \quad 0 \text{ for } X \leq 0$$

This equation describes the concentration of spores deposited at a given point (d) as a function of wind speed (s), the infection intensity of the source plant (I), a constant relating I to the source concentration of spores (k), and the coordinates of the point (X, Y), relative to the source. The coordinate system has the source at the origin, the X-axis parallel to the wind direction (with wind flowing in a positive direction), the Y-axis perpendicular to the X-axis on the plane defined by the ground (assumed to be flat), and the vertical Z-axis orthogonal to the X and Y axes. The shape of the three dimensional spore plum emanating from the source is defined by s , along with constants specifying the falling velocity of spores (W_s), the height of the source (H), and the standard deviation of spore dispersion (σ^2) in the horizontal and vertical directions. Following Levine and Okubu [20], we calculate σ^2 as a function of a diffusion coefficient, A , assuming that the variance in spore distribution increases linearly with time. We also assume that spores diffuse along the Y and Z axes at equal rates.

$$\sigma^2 = \frac{2AX}{s}$$

We fit the parameters k , W_s , and σ by minimizing the sum of squared differences between model predictions and the spore deposition data we collected from the spore traps arranged around diseased focal plants. We assumed that all spores deposited on a spore trap originated from the associated diseased focal plant, and that spore deposition occurred between noon on the day of deployment and ceased at noon two days post-deployment as the spore traps lost their adhesive properties and became saturated with dust. When predicting spore deposition using the TGPM, we set I equal to the infection intensity of the source diseased focal plant on the day of spore trap deployment and H equal to half of the maximum height of the source diseased focal plant at the day of spore trap deployment. As wind speed and direction were recorded every five minutes, we calculated total spore deposition as the sum of predicted deposition across five minute time windows. In each individual estimate, we set s equal to wind speed and calculated X and Y from wind direction and the location of the spore trap relative to the focal diseased plant (see Supplementary Text 3).

Transmission

Using the fitted TGPM we next sought to connect patterns of spore dispersal to transmission. The first step in this process was to align data on the spatiotemporal spread of disease with the locations of all healthy and infected plants (including non-focal plants) within each transect so that the relative locations of these could be determined (see Supplementary Text 4). Next, we built a dataset (‘corrected plant height data’) describing the height of all plants on the dates of epidemiological surveys. We preferentially used actual measurements from the initial population survey, epidemiological surveys, or focal plant measurements where they existed. For the

majority of plants, height was not recorded on the date of a given epidemiological survey. In these cases, we fore- or hind-casted height from a previous or future record of plant height (whichever was closer) using the fitted generalized additive model of plant growth and observed weather data spanning the period between the date of interest and the closest record. We included the random effect of study site when making predictions, but not the random effect of plant identity as we wished to forecast heights of both focal and non-focal plants and only focal plant data was used to fit the model of plant growth.

After completing these steps, we next built a ‘transmission data set’ connecting weather conditions, predicted spore deposition, plant height, and infection occurrence across the time windows between each epidemiological survey. We included separate entries for each healthy plant (focal and non-focal) during each time window. We limited the scope of this data to the periods for each transect in which all infected plants (that were not seedlings) were tracked as diseased focal plants. Using noon on the date of one epidemiological survey as the beginning of the time window, and noon on the date of the next epidemiological survey as the end of the time window, we extracted the same weather variables as described in ‘*Plant growth and within-host disease spread*’. Plant height values were extracted from the ‘corrected plant height’ data set. We determined that a plant became infected if a plant with identical coordinates (after data alignment) was recorded as newly infected in the epidemiological survey that took place at the end of the observation period. Total spore deposition for each healthy plant was calculated as the sum of predicted spore deposition originating from each of the plants in the same transect that was infected at the beginning of the time window. To predict the spore deposition experienced by a healthy plant that originated from an individual diseased plant, we used the TGMP. We extracted the height of the diseased plant at the beginning of the time window and set H to half

of this value. Likewise, we extracted the infection intensity of the diseased plant at the beginning of the time window, and set I equal to this value. For diseased seedlings that were not tracked as diseased focal plants, we set $I=0.1$, as most were observed to be lightly infected. There were 14 instances of missing infection intensity data for a diseased focal plant on a certain date. In these cases, we fore- or hind-casted infection intensity for that plant using the generalized additive model of infection intensity progression fit in '*Plant growth and within-host disease spread*' (while including the random effect for study site, but not for plant identity), the closest observation of infection intensity for that plant, and the weather data spanning the time period from the missing observation to the closest measurement. We set the values of k , W_s , and σ to those fit in the '*spore deposition*' section above. As wind speed and direction were recorded every five minutes, we summed spore deposition over five minutes windows that matched the resolution of the wind speed and direction data. For each of these windows, we predicted spore deposition after setting the values of X and Y based on the relative location of the healthy and diseased plants and the direction of the wind (see Supplementary Information).

Using this data set, we modeled infection outcomes in a generalized additive model framework. We assumed a binomial response distribution, coding the outcome of an infection as 1 and the outcome of no infection as 0. To account for variation in the duration of observation period, we used a complementary log-log link function, and included the log time as an offset in the model [44]. We included smooth terms for all weather variables as predictors, along with smooth terms study site and plant identity as random effects (accomplished by setting `bs="re"` in the `s()` function in `mgcv`). To infer the connection between spore deposition and infection, we also included a full tensor smooth product between the base 10 logarithm of mean total spore deposition per day and maximum plant height. When fitting the tensor, we fixed the smoothing

parameter for the marginal smooth for $\log_{10}$ total spore deposition to $1 * 10^{10}$. This effectively sets the shape of this marginal smooth to be linear, preventing any overfitting involving non-monotonic effects of spore deposition. Height was included as a factor in the tensor for two reasons. First, we calculated spore deposition for a point, but in reality the spore deposition experienced by a plant depends on the size of the ‘target’ that it presents. As such, larger plants are presumably challenged by a greater number of spores than a smaller plant for the same level of predicted spore deposition. Secondly, plant size could be correlated with quantitative resistance. To achieve computational feasibility, we fit this model using the `bam()` function in the `mgcv` R package and the fast restricted maximum likelihood parameter estimation method (“fREML”). We again implemented variable selection so that insignificant predictor terms would be effectively removed from the models via the double penalty approach.

To infer the effects of climate change and to illustrate the relationships between spore deposition, plant height, and infection risk, we predicted odds of infection using the estimated parameters of the fitted GAM (Fig. 5, Extended Data Figs. 9,10) and various weather data sets. In all of these projections, we included the random effect of site but excluded the random effect of plant identity.

Epidemiological model

Model description

To predict how the population level epidemiological dynamics of flax-rust might be affected in various climate change scenarios, we constructed a spatio-temporal epidemic model. This model tracks the infection status, infection intensity, and height of plants over time as infection spreads within the modeled population. There are three components required to simulate the model: 1)

initial conditions describing the locations and starting states of all plants, 2) weather data, and 3) models of spore dispersal, transmission, infection intensity progression, and plant growth.

The initial conditions for individual simulations of the epidemic model were in all cases constructed to represent either the BT or GM study populations on the date of an early epidemiological survey. For BT, this date was chosen as the first which had complete weather data (spanning 00:00:00 to 24:00:00), which was 6/24/2020. For GM, this date was set to 7/2/2020. Weather data was available for earlier dates, but a sharp increase in prevalence occurred during the prior week, perhaps due to prior missed observations of new infections. The model consistently underestimated observed prevalence when initialized prior to this increase in prevalence. We did not use the HM study population to initialize the model as no epidemiological surveys occurred on a date with complete weather data. Likewise, we do not use the CC study population to initialize the model because not all regions of the transect were mapped (see *Population mapping* sub-section in *Data collection* above).

The construction of initial conditions to represent a given site proceeded as follows. The locations of all plants were taken from the ‘corrected plant height’ data set. Plants observed to be infected on the date of the first epidemiological survey were initialized as infected, and all other plants were initialized as uninfected. Following the first epidemiological survey, all infected plants (except for several that were approximately 5cm or less in height) were designated diseased focal plants and their infection intensity was recorded. We used these measurements as the initial infection intensities of infected plants. We fore- or hind-casted infection intensities for any missing records using the method described in the transmission sub-section of the statistical analyses section above. Plant heights were taken from the ‘corrected plant height’ data set.

The weather data component of the model is used to predict spore dispersal, transmission, infection intensity progression, and plant growth in conjunction with the fitted statistical models of these processes. As such, it needs to describe mean weather conditions over the period spanning a simulation step. Additionally, because the TGPM is calibrated to make predictions over five minute intervals, the weather data must also describe average wind speed and direction over five minute windows.

We used the fitted statistical model relating weather to the processes spore dispersal (TGPM), transmission, infection intensity progression, and plant growth to simulate these processes in the epidemiological model. These models contain random effects for both site and plant identity. The random effect of plant identity was excluded when making predictions about infection intensity progression and plant growth as random effects were only fit for focal plants. We included random effects of plant identity when making predictions using the transmission model because random effects were fit for all plants in the modeled populations.

Using these components, the model simulates changes in infection status, infection intensity and plant height in time steps of seven days. The simulation for each time step proceeds as follows:

- (1) First, the model predicts the number of spores deposited at the location of each healthy plant over the course of the entire time step. As all infected plants are assumed to be sources of spore deposition, the model predicts total spore deposition as the sum of spore deposition originating from each infected plant. The spore deposition originating from a single infected plant is calculated as the sum of predictions over five minute windows spanning the time step. Individual predictions were generated using the TGPM with I is set to the infection intensity

of the source plant at the beginning of the time step, H set to 0.5 times the height of the source plant at the beginning of the time step, s set to average wind speed, and X and Y set according to locations of the source and target plants and wind direction (see Supplementary Information). The parameters k , W_s , and σ were set to the values fit to spore trap data.

(2) Next, for each healthy plant, the model infers the probability of that plant becoming infected from predicted spore deposition at that plant's location and mean weather metrics using the transmission model. Following this, a random number in $[0,1]$ is drawn, and if it is equal or lower to the probability of infection that plant becomes infected. All newly infected plants are set to have an infection intensity of 1.0.

(3) After simulating the transmission process, the model next simulates changes in infection intensity. For each plant, we simulated a prediction of the change in infection intensity per day by drawing randomly from the posterior of the infection intensity progression model. We used the infection intensity and height of that plant at the beginning of the time step along with mean weather metrics spanning the time step as the model inputs. We multiplied the predicted change in infection intensity per day by the length of the time step and added it to the plant's infection intensity at the beginning of the time step to calculate the new infection intensity of the plant.

(4) Finally, we used a similar process to simulate change in plant height. For each plant, we simulated a prediction of the change in height per day by drawing randomly from the posterior of the plant growth model. Again, we used

the height of that plant at the beginning of the time step along with mean weather metrics spanning the time step as model inputs. To calculate the new height of the plant, we multiplied the predicted change in height per day by the length of the time step and added this number to the plant's height at the beginning of the time step.

There are two sources of stochasticity within the epidemiological model. The first of these sources pertains to the probability of infection. Although the predicted probability of infection for a certain combination of predictor values is always the same, we compare this probability to a random number to determine if infection occurs. The second source of stochasticity pertains to predicted changes in infection intensity and plant height. We predict rates of infection intensity change and plant growth by drawing randomly from the posterior distributions of the corresponding fitted models. These sources of stochasticity allow us to understand the variation in epidemiological model predictions via repeated simulation.

Simulation

To validate the epidemiological model, we simulated 10 epidemiological trajectories for the BT and GM sites and compared the results to our observations of flax-rust spread to ensure a qualitative match. For each of these sites, we initialized the model using data corresponding to the site as described above and used observed weather (from the same site) data to run the simulation. When extracting weather variables from observed weather data sets, we defined the observation period as 00:00:00 on the first day in a step to 00:00:00 on the last day in a step (e.g. noon on July 1st to noon on July 8th). We included the random effects of site and plant identity

when making predictions from the transmission model, and the random effects of site when making predictions from the infection intensity and plant growth models.

To infer the effects of climate change of flax-rust epidemiology, we again initialized the model using data corresponding to the for BT and GM sites, and then simulated 10 epidemiological trajectories for each set of weather data (RCP4.5 and 8.5 emissions scenarios and the years 2020, 2030, 2040, 2045, 2050, 2060 and 2070). We extracted weather metrics as described in the *Weather data* subsection of the *Data collection* section above. We used observed records of wind speed and direction to simulate spore deposition using the TGPM as the projected data sets lack these measures. As before we included the random effects of site and plant identity when making predictions from the transmission model, and the random effects of site when making predictions from the infection intensity and plant growth models. For each set of 10 model simulations corresponding to a unique site and weather data set combination, we used the same 10 random number seeds.

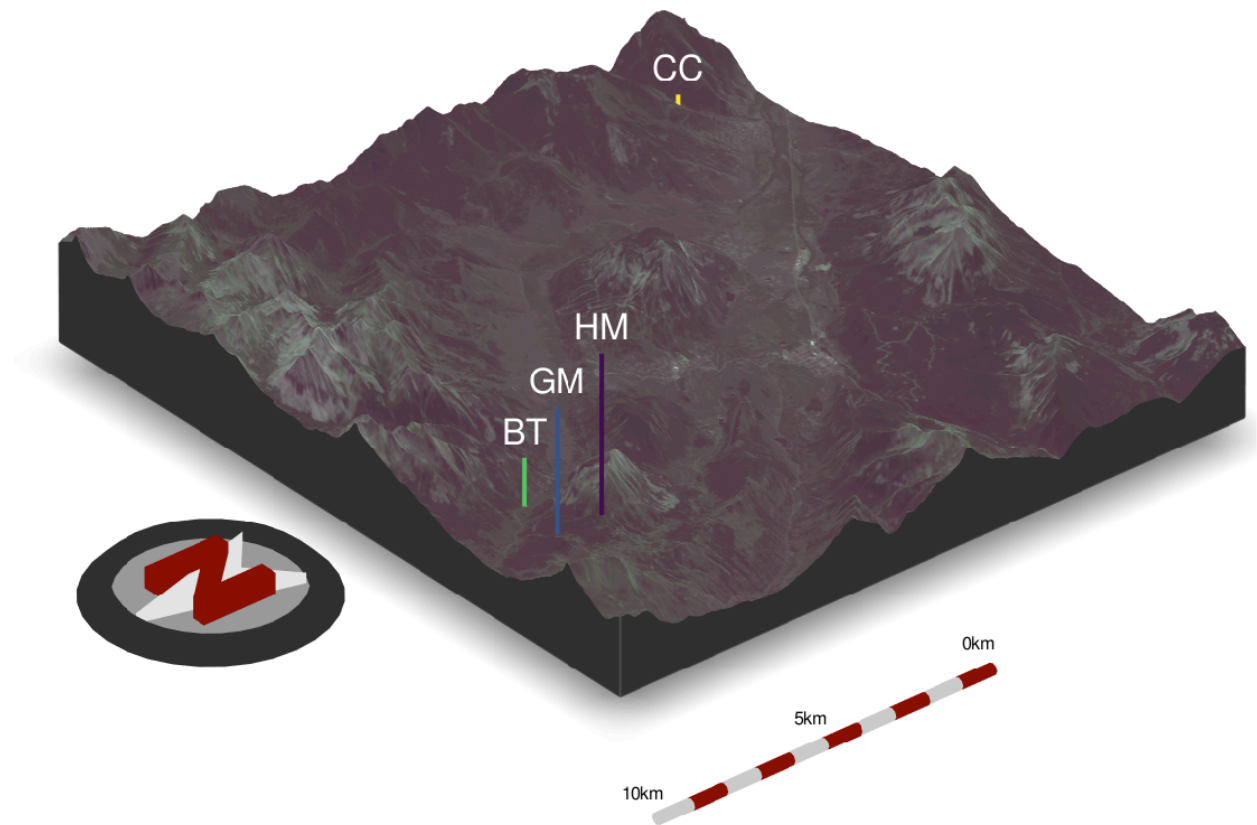
Acknowledgements

This research was supported by grants from the Princeton University High Meadows Environmental Institute, the Princeton University Center for Health and Wellbeing, and the Rocky Mountain Biological Laboratory. IFM is supported by a NSF Graduate Research Fellowship.

We would like to thank Jennie Reithel and the Rocky Mountain Biological Laboratory for research and logistical support, and Emily Miller for help with data collection.

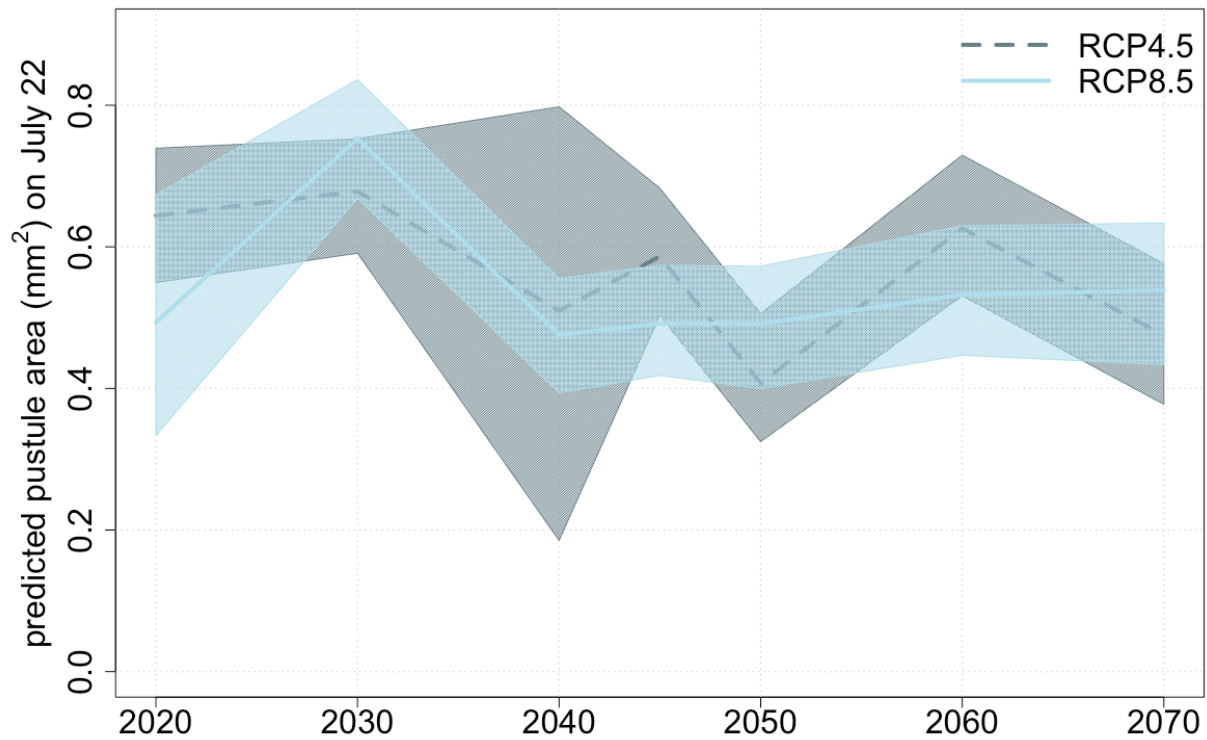
We acknowledge the World Climate Research Programme's Working Group on Coupled Modeling, which is responsible for CMIP, and we thank the National Science Foundation, Department of Energy and National Center for Atmospheric Research modeling group for producing and making available their model output. For CMIP the U.S. Department of Energy's Program for Climate Model Diagnosis and Intercomparison provides coordinating support and led development of software infrastructure in partnership with the Global Organization for Earth System Science Portals.

Extended Data Figures



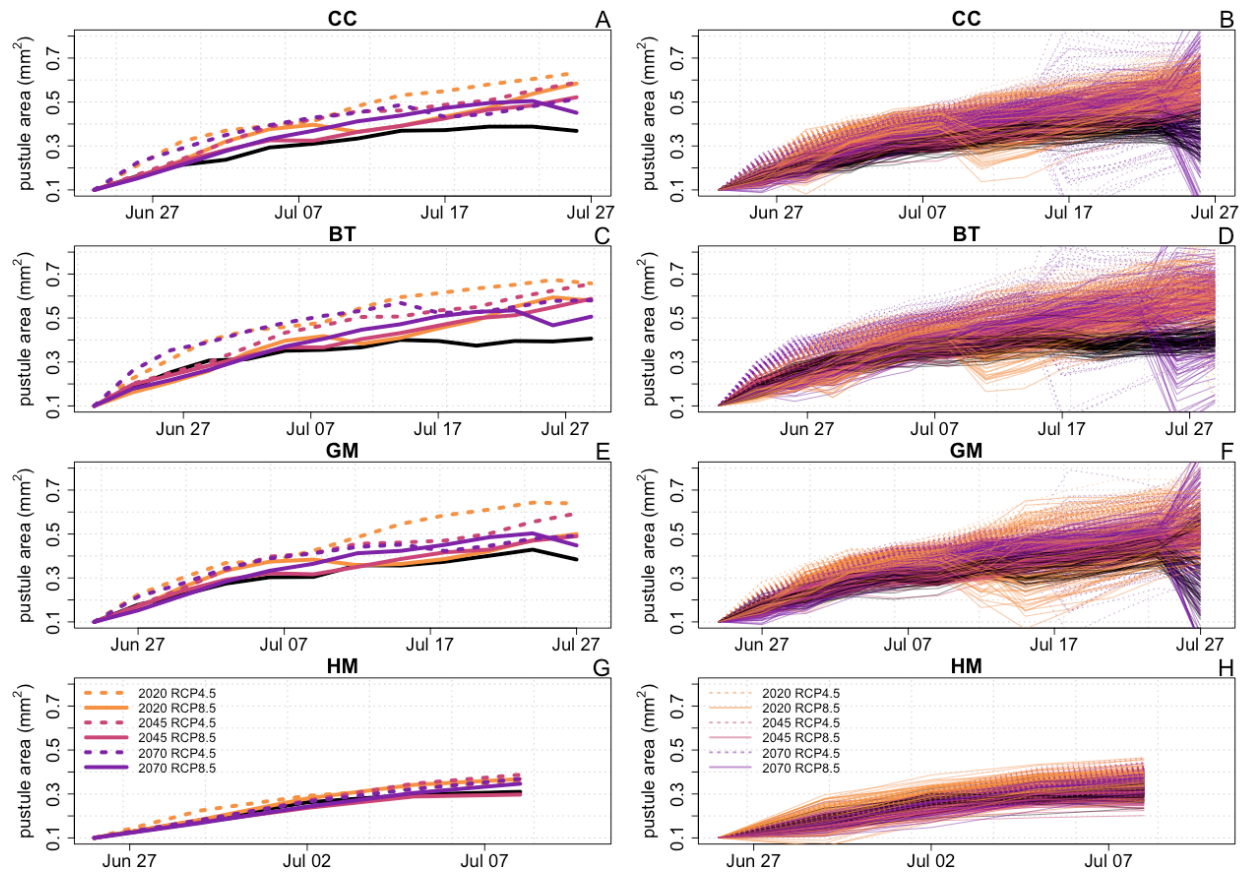
Extended Data Figure 1: Study site map

Vertical lines show the relative locations of the ‘Cement Creek’ (CC), ‘Bus Turnaround’ (BT), ‘Gothic Mountain’ (GM), and ‘High Meadow’ (HM) study sites. Elevation is not to scale.



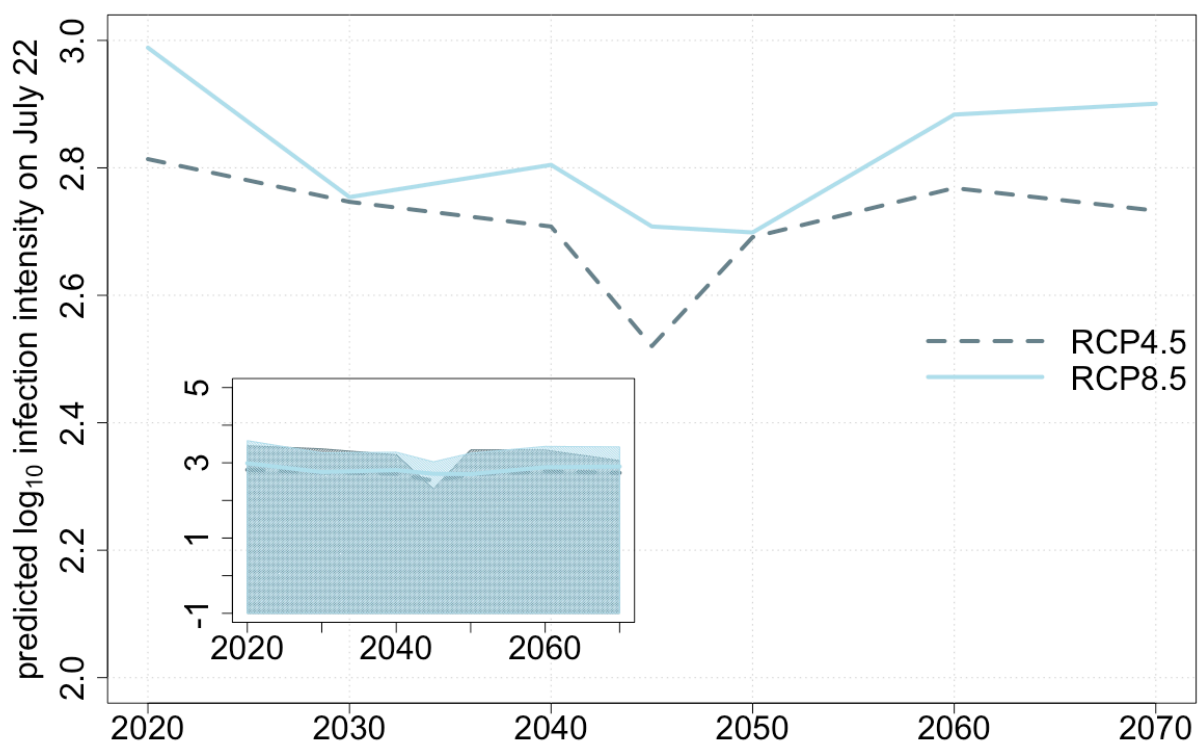
Extended Data Figure 2: Patterns of end-of-simulation pustule area across future climate scenarios

We simulated 100 trajectories of pustule growth (for a pustule starting with area 0.1 mm²) at the GM site for each of 12 future weather data sets encompassing the RCP 4.5 and 8.5 emissions scenarios and the years 2020, 2030, 2040, 2045, 2050, 2060, and 2070. Lines show the mean predicted pustule area at the end of the simulation for each year and each emissions scenario. Shaded regions span the 10% to 90% quantiles of predictions.



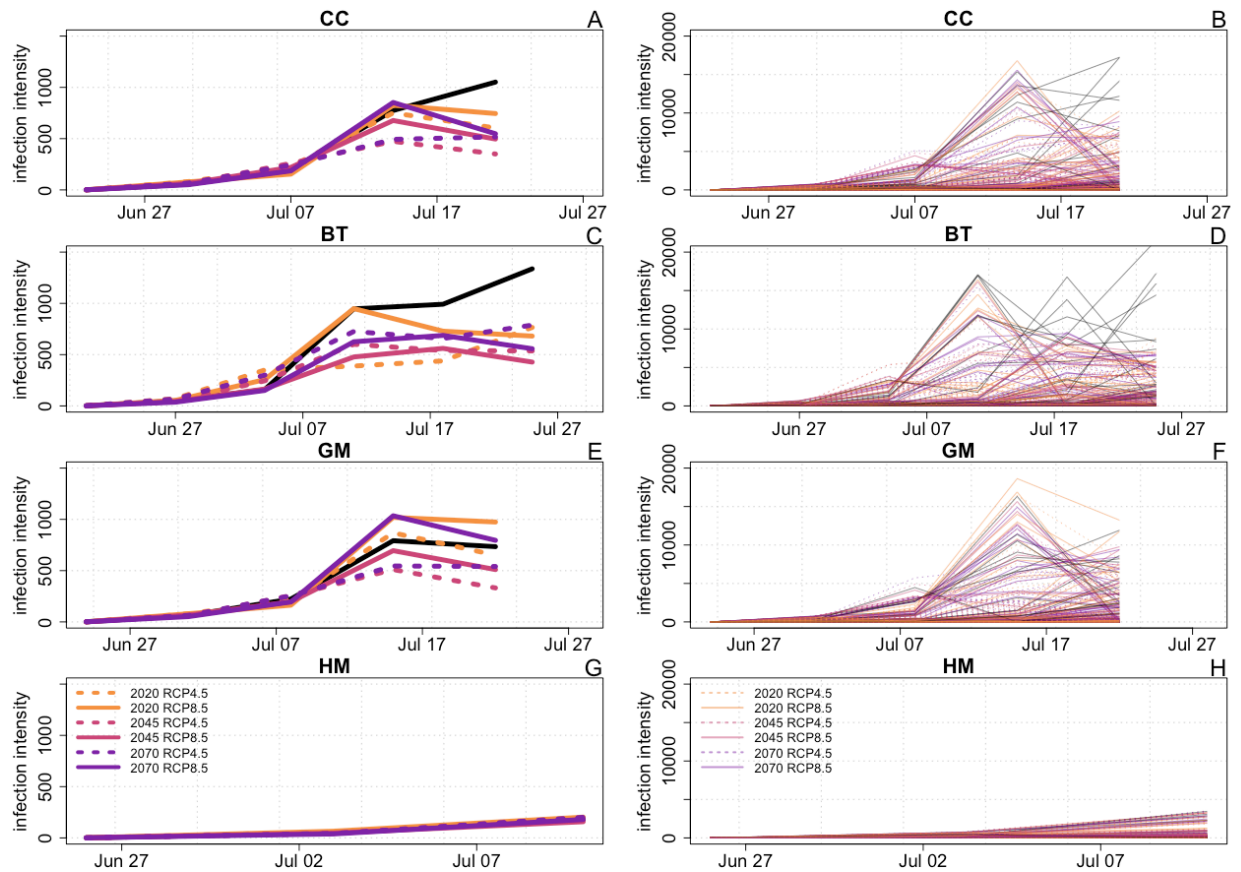
Extended Data Figure 3: Simulated effects of future climate on pustule growth across study sites

Using the fitted GAM model, we 100 simulated trajectories of pustule growth (for a pustule starting with area 0.1 mm^2) at the four study sites for each of six future weather data sets. Lines in panels A, C, E, and G show the mean of the trajectories simulated for each weather condition. Lines in panels B, D, F, and H show individual trajectories. Panels A and B correspond to the CC site, panels C and D correspond to the BT site, panels E and F correspond to the GM site (panel E is identical to Fig. 2J), and panels G and H correspond to the HM site.



Extended Data Figure 4: Patterns of end-of-simulation infection intensity across future climate scenarios

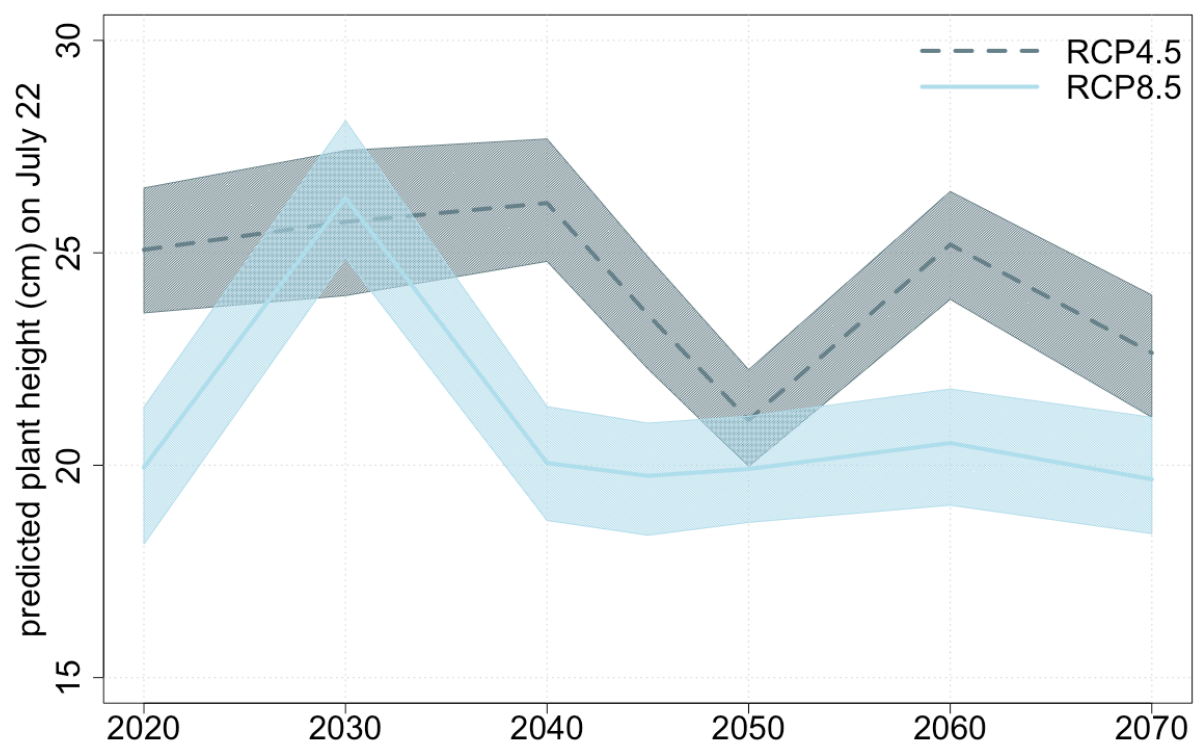
We simulated 100 trajectories of infection intensity (for a 25cm tall plant starting with infection intensity 1) at the GM site for each of 12 future weather data sets encompassing the RCP 4.5 and 8.5 emissions scenarios and the years 2020, 2030, 2040, 2045, 2050, 2060, and 2070. Lines show the mean predicted infection intensity at the end of the simulation for each year and each emissions scenario. The inset plot shows the same results, with the range of the vertical axis expanded. Shaded regions span the 10% to 90% quantiles of predictions.



Extended Data Figure 5: Simulated effects of future climate on infection intensity

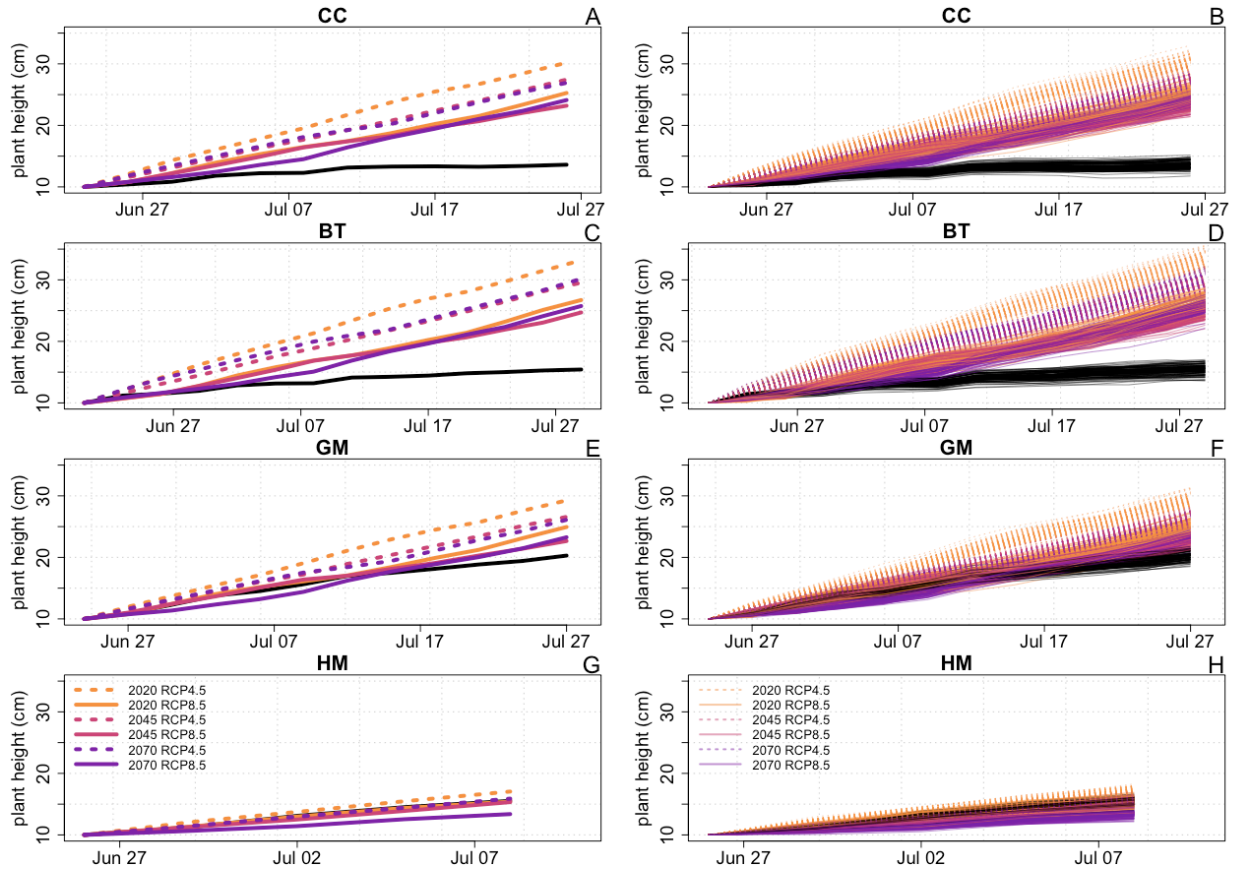
progression across study sites

We simulated 100 trajectories of infection intensity progression (for a plant starting with a height of 25 cm and infection intensity of 1) at each site for six future weather data sets using the fitted GAM. Lines in panels A, C, E, and G show the mean of the trajectories simulated for each weather condition. Lines in panels B, D, F, and H show individual trajectories. Panels A and B correspond to the CC site, panels C and D correspond to the BT site, panels E and F correspond to the GM site (panel E is identical to Fig. 3J), and panels G and H correspond to the HM site.



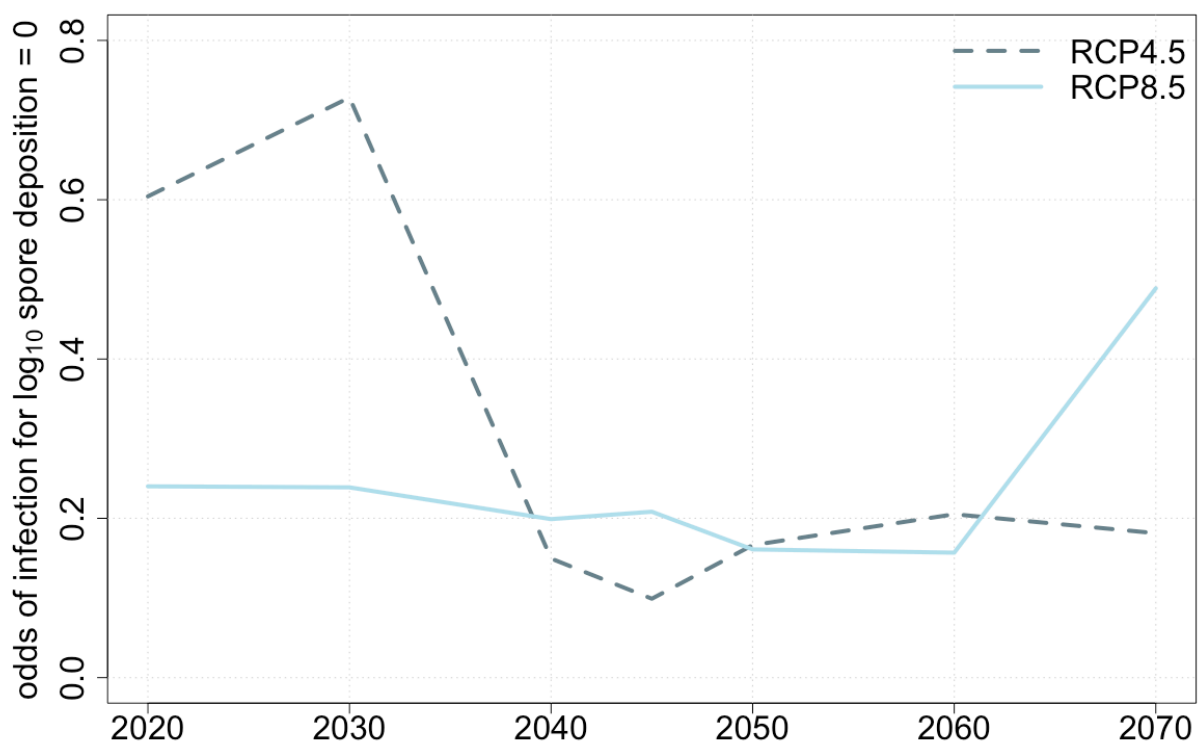
Extended Data Figure 6: Patterns of end-of-simulation plant height across future climate scenarios

We simulated 100 trajectories of plant growth (for a 25cm tall uninfected plant) at the GM site for each of 12 future weather data sets encompassing the RCP 4.5 and 8.5 emissions scenarios and the years 2020, 2030, 2040, 2045, 2050, 2060, and 2070. Lines show the mean predicted plant height at the end of the simulation for each year and each emissions scenario. Shaded regions span the 10% to 90% quantiles of predictions.



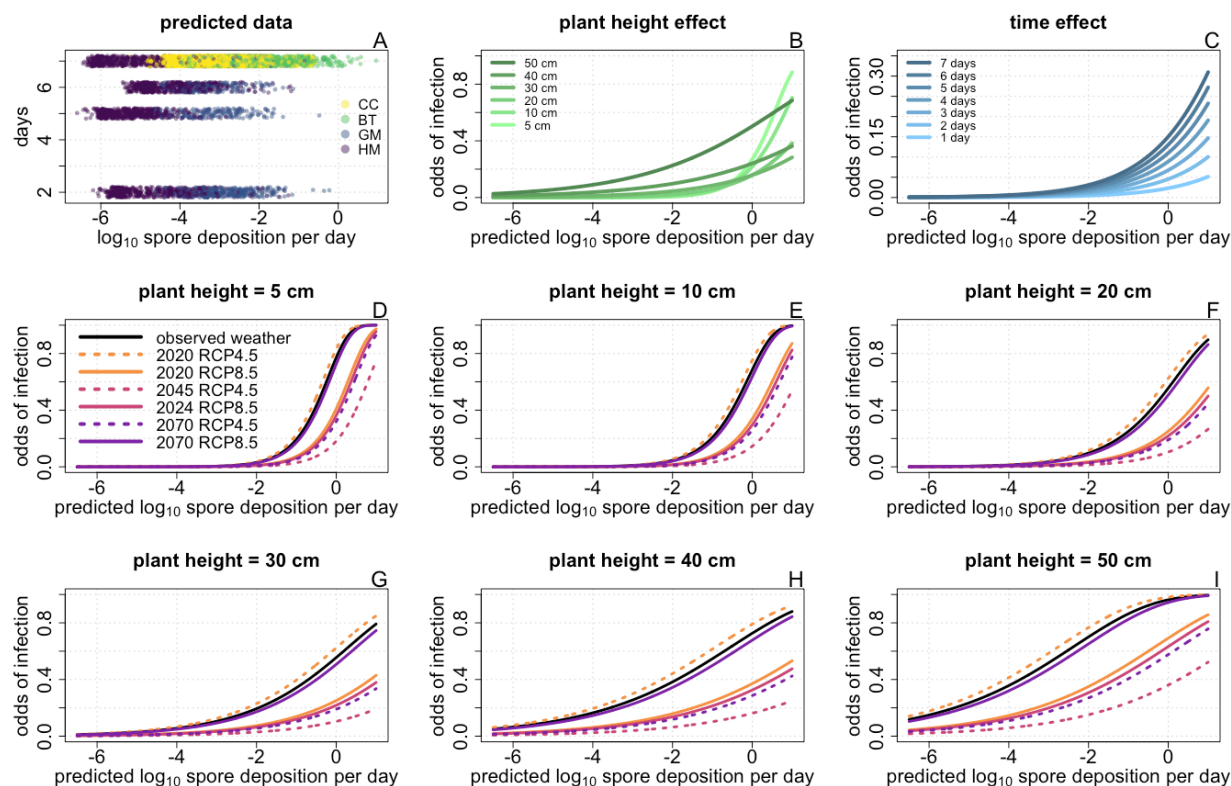
Extended Data Figure 7: Simulated effects of future climate on plant growth

We simulated 100 growth trajectories (for a plant starting with a height of 10 cm) at each site for six future weather data sets using the fitted GAM. Lines in panels A, C, E, and G show the mean of the trajectories simulated for each weather condition. Lines in panels B, D, F, and H show individual trajectories. Panels A and B correspond to the CC site, panels C and D correspond to the BT site, panels E and F correspond to the GM site (panel E is identical to Fig. 4J), and panels G and H correspond to the HM site.



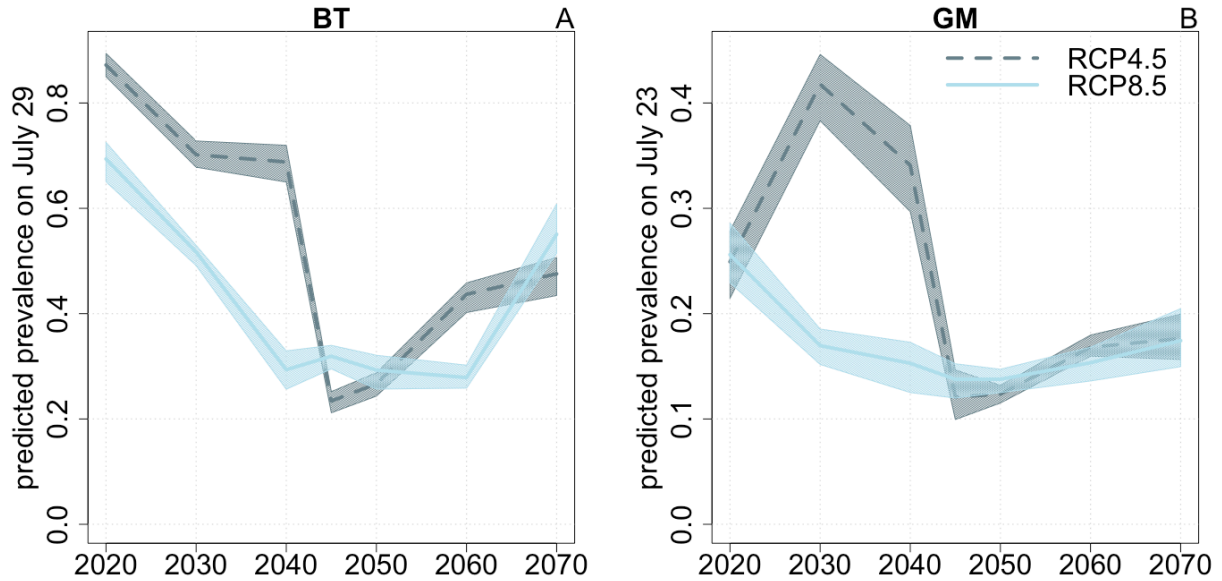
Extended Data Figure 8: Patterns of predicted infection odds across future climate scenarios

We simulated the odds of infection over the time period spanning July 7th-July 14th for each of 12 future weather data sets encompassing the RCP 4.5 and 8.5 emissions scenarios and the years 2020, 2030, 2040, 2045, 2050, 2060, and 2070. In all cases, we considered a plant with height 25cm at the GM site and assumed $\log_{10}$ spore deposition per day = 0. Lines show the predicted odds of infection for each year and each emissions scenario.



Extended Data Figure 9: The effects of plant height and time on transmission odds

Panel A shows an alternate visualization of the predicted spore deposition data presented in Fig. 5A, with the length of the period between observations of plant infection status plotted against $\log_{10}$ predicted spore deposition per day. Panels B and C show how the odds of infection inferred by the fitted GAM relate to plant height and observation time respectively. Predictions in panels B and C were generated using weather data observed at the GM site between July 7th and July 14th. Panels D-I show the relationship between $\log_{10}$ predicted spore deposition per day, plant height and the odds of infection inferred for different future climate scenarios. Plant heights are 5, 10, 20, 30, 40, and 50cm in panels D-I respectively. All predictions were generated for the GM site.



Extended Data Figure 10: Patterns of end-of-simulation infection prevalence across future climate scenarios

Using the spatiotemporal epidemiological model, we simulated 10 epidemics at the BT (panel A) and GM (panel B) sites for each of 12 future weather data sets encompassing the RCP 4.5 and 8.5 emissions scenarios and the years 2020, 2030, 2040, 2045, 2050, 2060, and 2070. Lines show the mean predicted prevalence at the end of the simulation for each year and each emissions scenario. Shaded regions span the 10% to 90% quantiles of predictions.

Supplementary information

Supplementary Text 1: Environmental condition monitoring equipment

We recorded temperature and humidity using HOBO Temperature/RH data loggers (part #: MX2301A) enclosed in solar radiation shields. These loggers were mounted approximately 30cm above ground level on wooden stakes. We recorded rainfall, wind speed, and wind direction using sensors connected to HOBO Micro Stations (part # H21-USB). Rainfall was measured using Davis (0.2 mm) Rain Gauge Smart Sensors (part # S-RGF-M0002). Wind speed and direction were measured using Davis Wind Speed and Direction Smart Sensors (part #: S-WCF-M003).

Supplementary Text 2: Absolute humidity calculation

To calculate absolute humidity (in terms of g H₂O per cubic meter of air) from our observations of temperature and humidity, we began with the ideal gas law where P is pressure, V is volume, n is the number of gas molecules in mols, R is the universal gas constant, and T is the temperature in Celsius.

$$PV = nR(T + 273.15) \quad (s1)$$

By setting $V = 1$, substituting in the value of R , and rearranging variables, we can solve for n :

$$n = \frac{P}{0.08314(T + 273.15)} \quad (s2)$$

In order for n to represent the number of mols of water present in the gas, we need to substitute the partial vapor pressure of water, p_w , for P . We calculate the partial pressure of water by

multiplying that saturation vapor pressure of water, p_{ws} , given by Tetens's formula [45] by the relative humidity, ϕ , expressed as a percentage:

$$p_{ws} = 6.112 e^{\frac{17.67 \cdot T}{T + 243.5}} \quad (s3)$$

$$p_w = p_{ws} \frac{\phi}{100} \quad (s4)$$

By substituting s3 and s4 into s2, we obtain the number of mols of water in 1 cubic meter of air as:

$$n = \frac{6.112 e^{\frac{17.67 \cdot T}{T + 243.5}} \phi}{0.08314 (T + 273.15) 100} \quad (s5)$$

By multiplying the right side of this expression by the molecular weight of water, 18.02g/mol, and simplifying, we finish our derivation of absolute humidity as a function of temperature and relative humidity:

$$absolute\ humidity \left(g \frac{H_2O}{m^3\ air} \right) = \frac{13.24732 e^{\frac{17.67 \cdot T}{T + 243.5}} \phi}{(T + 273.15)} \quad (s6)$$

Supplementary Text 3: Calculating values of X and Y in the tilted gaussian plume model

To estimate spore deposition using the TGPM it is necessary to find the values of X and Y from wind direction and the coordinates of the source plant and target within the transect. Figure S4 gives a visual representation of this problem. This is accomplished as follows:

First, we define a coordinate system such that the corners of the transect are located at (0,0), (10,0), (0,20), (10,20). In the raw wind data, a wind direction (θ) of 0° or 360°

corresponded to north. We applied a site specific correction so that a corrected wind direction (θ^*) of 0° or 360° would point in the same direction as $\langle 0,1 \rangle$:

$$\theta^* = \theta - 332^\circ \text{ for site CC} \quad (s7a)$$

$$\theta^* = \theta - 75^\circ \text{ for site BT} \quad (s7b)$$

$$\theta^* = \theta - 309^\circ \text{ for site GM} \quad (s7c)$$

$$\theta^* = \theta - 40^\circ \text{ for site HM} \quad (s7d)$$

Let (x_S, y_S) and (x_T, y_T) be the coordinates of the source plant and target respectively. Using the direction of the wind, we can define a point (x_W, y_W) such that the line passing through (x_S, y_S) and (x_W, y_W) points in the direction of the wind:

$$x_W = x_S + \sin(\theta^*) \quad (s8a)$$

$$y_W = y_S + \cos(\theta^*) \quad (s8b)$$

For convenience, we name the line passing through (x_S, y_S) and (x_W, y_W) W . We wish to find the point (x_p, y_p) on W that is closest to (x_T, y_T) , so that X can be calculated as the length of the segment $\underline{X}$ connecting (x_S, y_S) and (x_p, y_p) , and Y can be calculated as the length of the segment $\underline{Y}$ connecting (x_p, y_p) and (x_T, y_T) . The coordinates of points on W can be written as

$$(x_S + x_W c, y_S + y_W c) \quad (s9)$$

where c is a scalar. We can define D , the squared distance between a point on W and (x_T, y_T) , as a function of c :

$$D(c) = (x_T - (x_S + (x_W - x_S)c))^2 + (y_T - (y_S + (y_W - y_S)c))^2 \quad (s10)$$

We next find the derivative of $D(c)$:

$$D'(c) = 2(x_T - (x_S + (x_W - x_S)c))(x_W - x_S) + 2(y_T - (y_S + (y_W - y_S)c))(y_W - y_S) \quad (s11)$$

We can find the value of c that can be used to calculate (x_p, y_p) by setting $D'(c) = 0$ and solving:

$$c = \frac{(x_T - x_S)(x_W - x_S) + (y_T - y_S)(y_W - y_S)}{(x_W - x_S)^2 + (y_W - y_S)^2} \quad (s12)$$

We can then use this value of c to construct the following equations for x_p and y_p :

$$x_P = x_S + x_W \left(\frac{(x_T - x_S)(x_W - x_S) + (y_T - y_S)(y_W - y_S)}{(x_W - x_S)^2 + (y_W - y_S)^2} \right) \quad (s13a)$$

$$y_P = y_S + y_W \left(\frac{(x_T - x_S)(x_W - x_S) + (y_T - y_S)(y_W - y_S)}{(x_W - x_S)^2 + (y_W - y_S)^2} \right) \quad (s13b)$$

The absolute values of X and Y can then be solved for as:

$$|X| = ((x_P - x_S)^2 + (y_P - y_S)^2)^{\frac{1}{2}} \quad (s14a)$$

$$|Y| = ((x_P - x_T)^2 + (y_P - y_T)^2)^{\frac{1}{2}} \quad (s14b)$$

Because the shape of the spore distribution predicted by the TGPM is always symmetrical, we can set $Y = |Y|$. However, spore deposition only occurs for $X > 0$, and as such it is necessary to determine the sign of X . We take advantage of the fact that the points (x_S, y_S) , (x_W, y_W) , and (x_p, y_p) all fall on W , and use the following set of rules to determine the sign of X :

s(15)

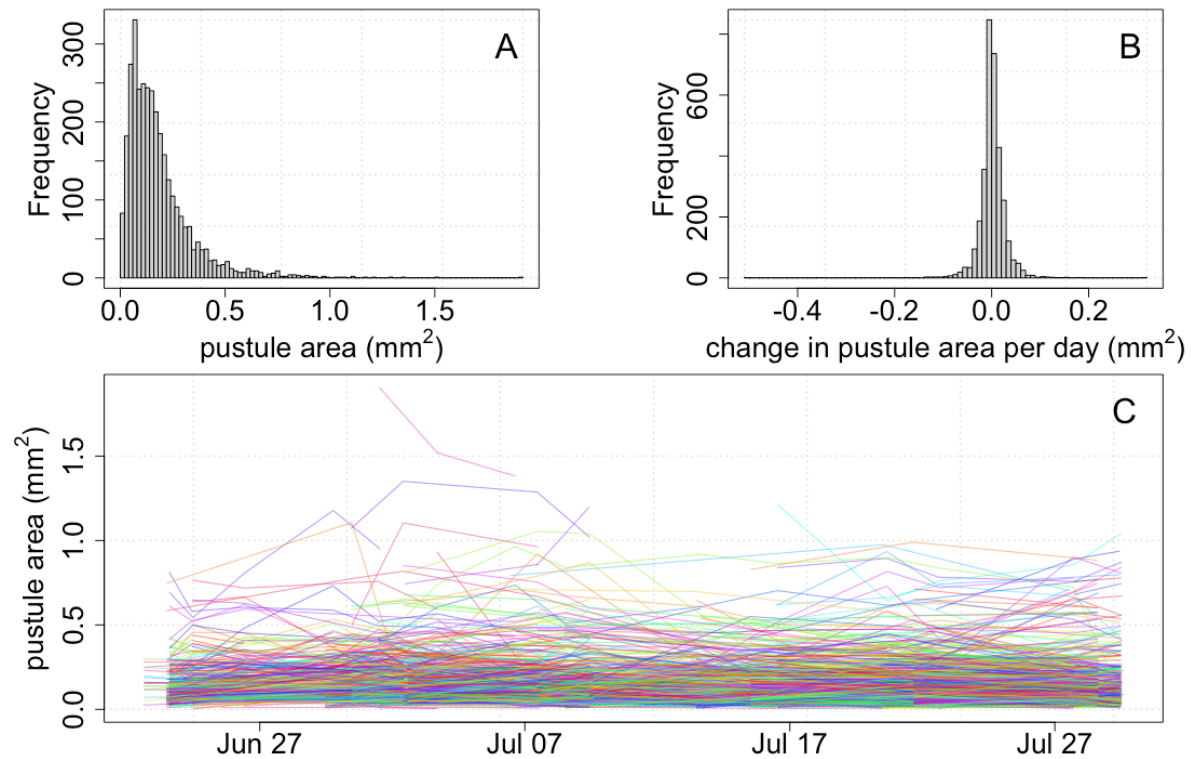
- *for* $x_W > x_S$
 - *for* $x_P > x_S, X > 0$
 - *for* $x_P < x_S, X < 0$
- *for* $x_W < x_S$
 - *for* $x_P > x_S, X < 0$
 - *for* $x_P < x_S, X > 0$
- *for* $x_W = x_S$
 - *for* $y_W > y_S$
 - *for* $y_P > y_S, X > 0$

- $for\ y_P < y_S, X < 0$
- $for\ y_W < y_S$
- $for\ y_P > y_S, X < 0$
- $for\ y_P < y_S, X > 0$

Supplementary Text 4: Plant location alignment

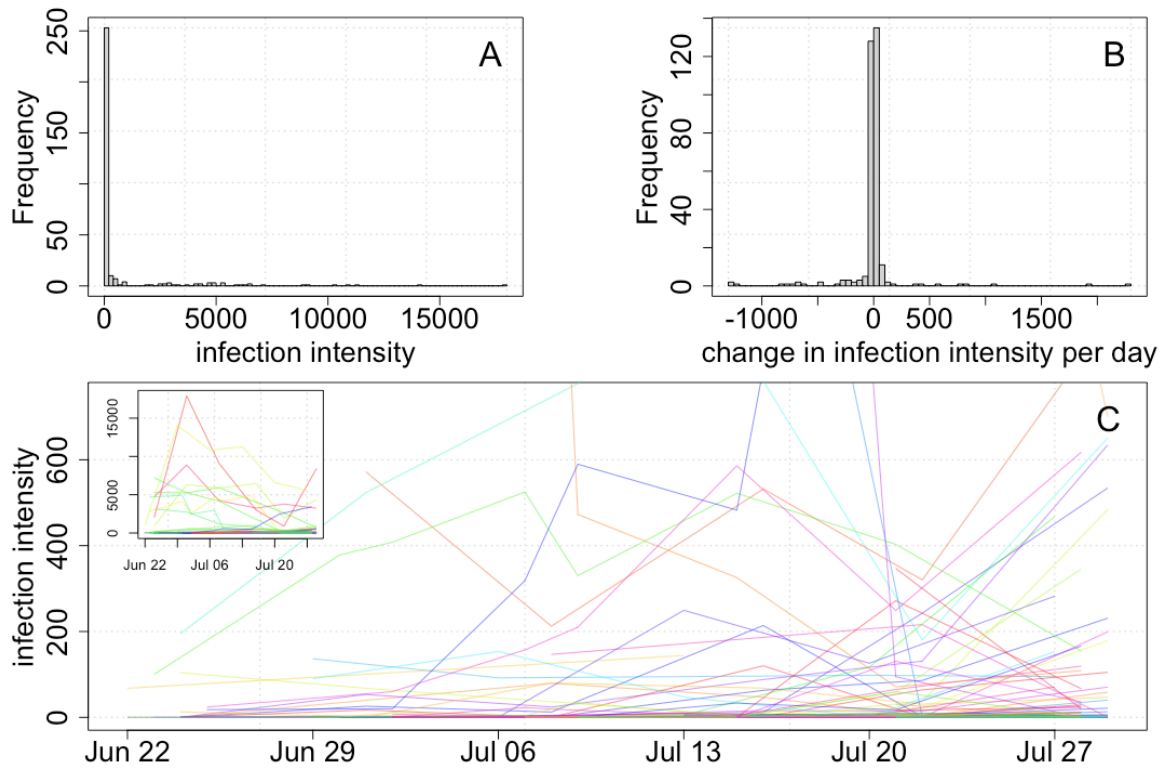
We corrected the coordinates of newly infected plants (recorded in ‘epidemiological surveillance’) so that they could be precisely location matched to previously uninfected plants (whose locations were recorded in ‘population mapping’). We expected slight differences between coordinates recorded during ‘epidemiological surveillance’ and ‘population mapping’ due to measurement error. Many newly infected plants were marked with plant tags, either because they were healthy focal plants, or because they were being tracked as a part of a concurrent demography study. We updated the coordinates of newly infected tagged plants to match those recorded for those same plants during ‘population mapping’. For newly infected untagged plants, this precise matching process was not possible. Instead, we matched these plants to the closest untagged, previously uninfected, and unmatched plant within 25cm, and updated their coordinates accordingly. If the newly infected plant was not able to be matched, then we did not correct its coordinates, but rather retroactively added a healthy plant with those same coordinates to the plant location dataset generated via ‘population mapping’. We added these records so that all infected plants would appear in data used to validate the epidemiological model, but we excluded these added records from our statistical analysis in the ‘*Transmission*’ section to avoid biasing our dataset towards infection events.

Supplementary figures



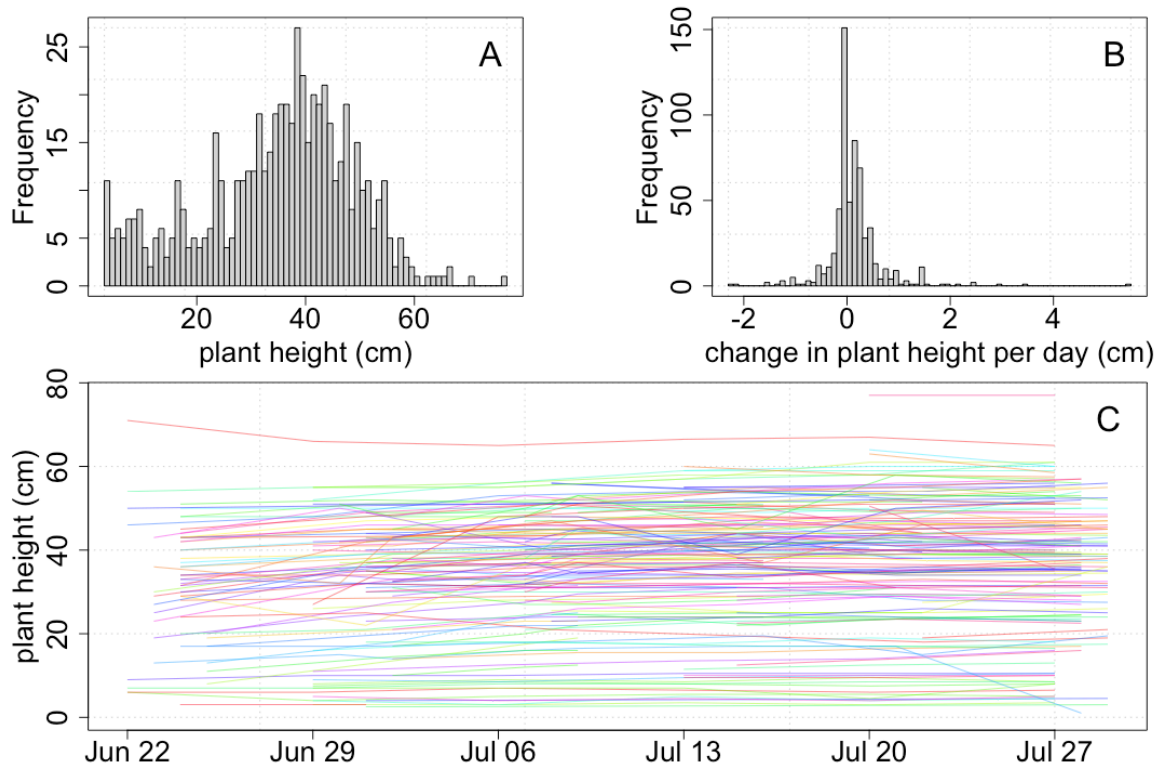
Supplementary Figure 1: Pustule area data

Panel A shows the distribution of pustule area measurements. Panel B shows the distribution of change in pustule area per day, the response variable in the GAM fit to infer climate effects on the replication rate of *M. lini*. Panel C shows the observed trajectories of pustule area, with each line representing an individual pustule.



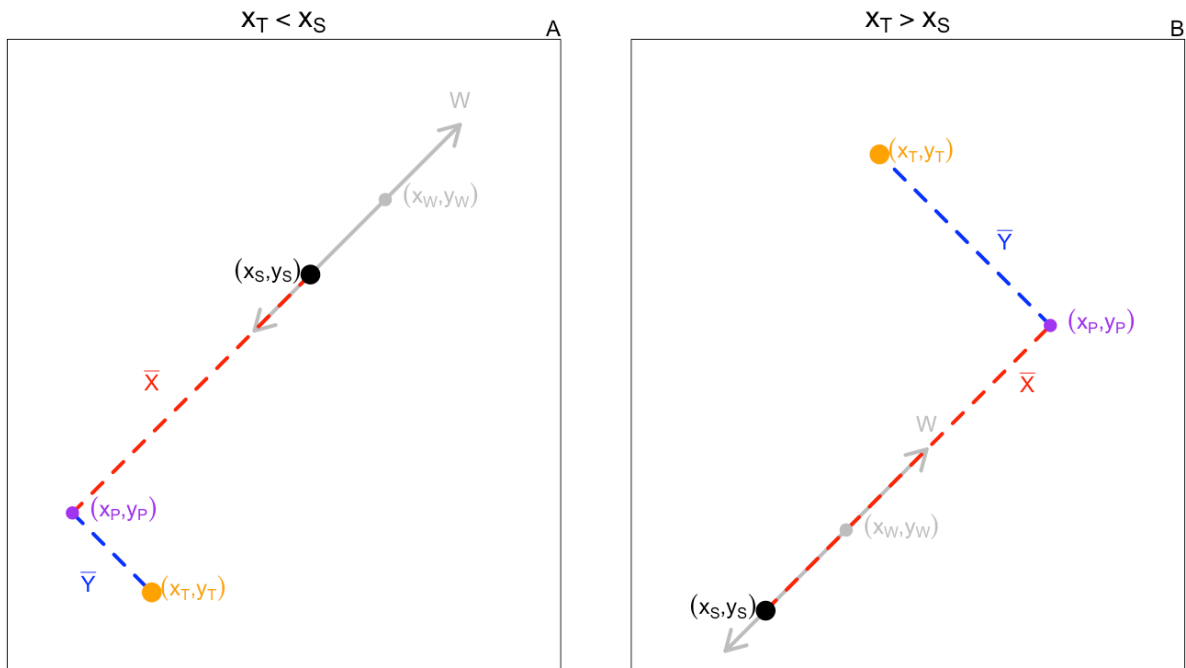
Supplementary Figure 2: Infection intensity data

Panel A shows the distribution of infection intensity measurements. Panel B shows the distribution of change in infection intensity per day, the response variable in the GAM fit to infer climate effects on infection intensity progression. Panel C shows observed trajectories of infection intensity, with each line representing an individual infected plant. The inset plot shows the same data with the y axis rescaled to show data for heavy infected plants. Colors are consistent across the main and inset plot.



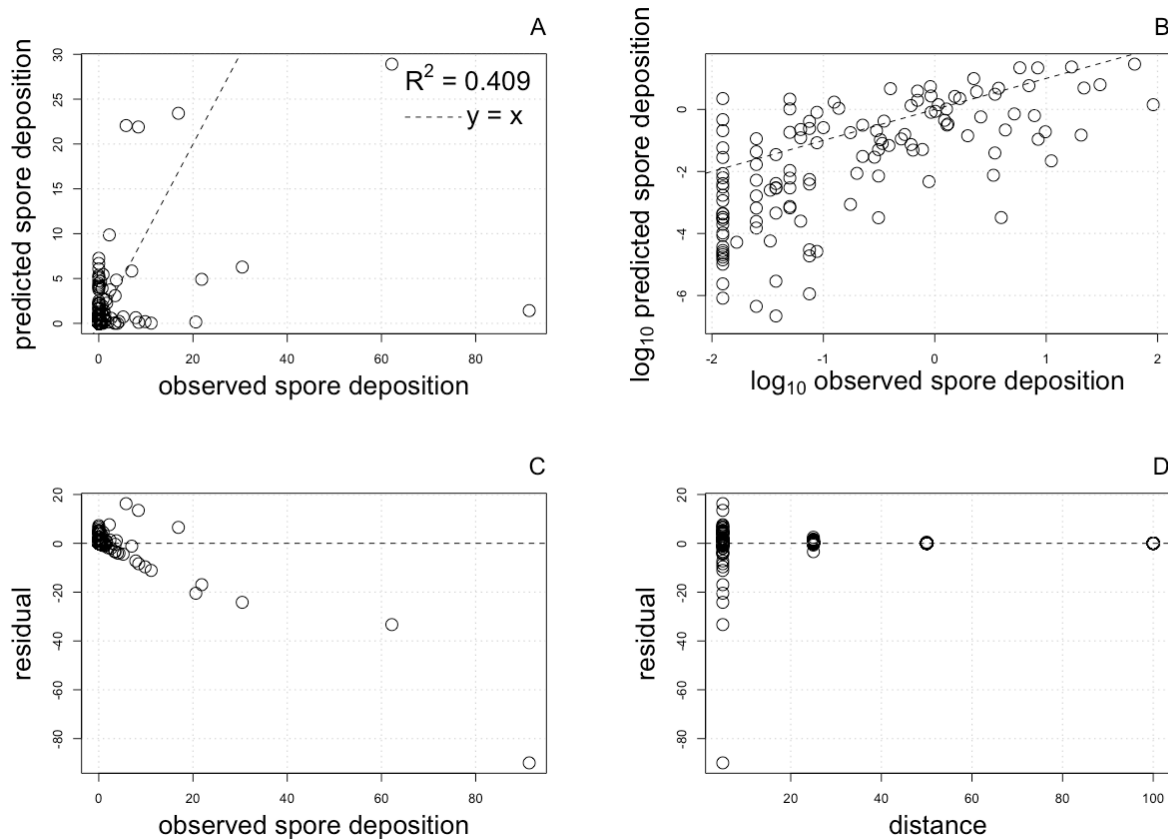
Supplementary Figure 3: Plant growth data

Panel A shows the distribution of pustule height measurements. Panel B shows the distribution of change in plant height per day, the response variable in the GAM fit to infer the effects of climate and infection on plant growth. Panel C shows observed height trajectories, with each line representing an individual plant.



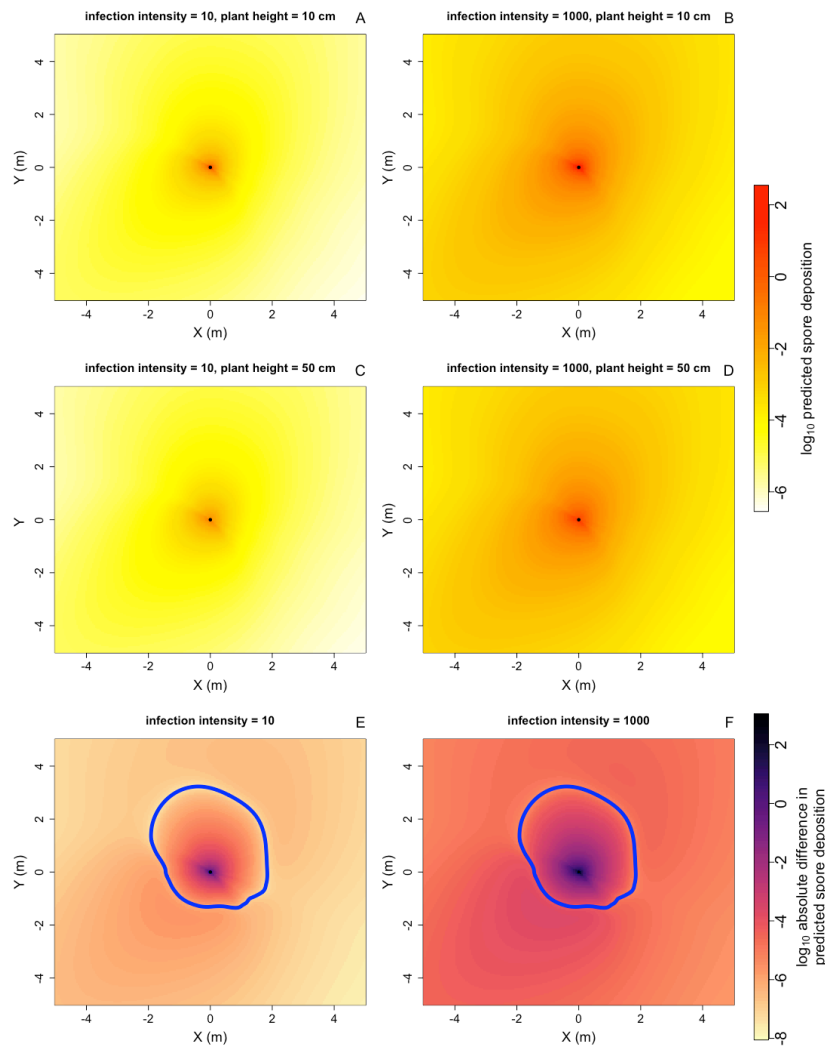
Supplementary Figure 4: Calculating values of X and Y in the tilted gaussian plume model

Figure S4 illustrates how the values of X and Y are found from the locations of the source and target plants and wind direction. Panel A shows an example where the target plant is to the left of the source plant, and panel B shows an example where the target plant is to the right of the source plant. Labels match the notation presented in the text.



Supplementary Figure 5: Tilted gaussian plume model diagnostics

Panel A shows predictions generated by the TGPM plotted against spore deposition measurements from spore traps. The model explained 40.9% of the variance in the observed data. Panel B shows the same data as panel A with a $\log_{10}$ transformation applied to both observed and predicted values to illustrate how the model does a reasonable job of predicting the correct magnitude of spore deposition. In panel C, observed spore deposition is plotted against model residuals to illustrate that model performance was acceptable across the majority of the distribution of observed spore deposition values. Panel D shows the model residual plotted against the distance between a spore trap and the associated diseased focal plant. The mean of residuals is approximately zero for all distances, indicating that the model performs equally well at predicting spore deposition over small and large distances. The dashed lines in panels A and B show the $y=x$ line. The dashed lines in C and D show $y=0$.



Supplementary Figure 6: The effect of source plant height and infection intensity on spatial patterns of spore distribution predicted by the tilted gaussian plume model

Figure S6 shows how the spatial pattern of spore distribution predicted by the TGPM depends upon the height and infection intensity of the source plant. In all panels, horizontal and vertical axes represent space. The source plant is located at $\{X=0, Y=0\}$. Predictions were generated using wind data collected at the GM site spanning noon on July 1st to noon on July 8th. In panels A and B, the plant's height is assumed to be 10cm, and in C and D, the plant's height is assumed to be 50cm. The plant is assumed to have infection intensity 10 in A and C, and infection intensity 1,000 in B and D. Colors in A-E show $\log_{10}$ predicted spore deposition. Panels E and F clarify the difference in patterns of spore distribution between panels A and C, and panels B and D. Colors show the base-10 logarithm of the absolute difference in predicted spore deposition for plants with height 10 cm and 50 cm. Infection intensity for both plants is assumed to be 10 in panel E and 1,000 in panel F. The blue line shows where the difference in total spore

deposition is 0. The area enclosed by the blue line receives more spore deposition when plant height is 10 cm than when plant height is 50 cm. The area outside of the blue line receives more spore deposition when plant height is 50 cm than when plant height is 10 cm.

References

1. Savary, S. *et al.* The global burden of pathogens and pests on major food crops. *Nat. Ecol. Evol.* **3**, 430–439 (2019).
2. Chaloner, T. M., Gurr, S. J. & Bebber, D. P. Plant pathogen infection risk tracks global crop yields under climate change. *Nat. Clim. Chang.* **11**, 710–715 (2021).
3. Ristaino, J. B. *et al.* The persistent threat of emerging plant disease pandemics to global food security. *Proc. Natl. Acad. Sci.* **118**, e2022239118 (2021).
4. McDonald, B. A. & Stukenbrock, E. H. Rapid emergence of pathogens in agro-ecosystems: global threats to agricultural sustainability and food security. *Philos. Trans. R. Soc. B Biol. Sci.* **371**, 20160026 (2016).
5. Boyd, I. L., Freer-Smith, P. H., Gilligan, C. A. & Godfray, H. C. J. The Consequence of Tree Pests and Diseases for Ecosystem Services. *Science* **342**, 1235773 (2013).
6. Mitchell, C. E. Trophic control of grassland production and biomass by pathogens. *Ecol. Lett.* **6**, 147–155 (2003).
7. Colhoun, J. Effects of Environmental Factors on Plant Disease. *Annu. Rev. Phytopathol.* **11**, 343–364 (1973).
8. Garrett, K. A., Dendy, S. P., Frank, E. E., Rouse, M. N. & Travers, S. E. Climate Change Effects on Plant Disease: Genomes to Ecosystems. *Annu. Rev. Phytopathol.* **44**, 489–509 (2006).
9. Juroszek, P. & von Tiedemann, A. Linking Plant Disease Models to Climate Change Scenarios to Project Future Risks of Crop Diseases: A Review. *J. Plant Dis. Prot.* **122**, 3–15 (2015).
10. Gilligan, C. A. & van den Bosch, F. Epidemiological Models for Invasion and Persistence

- of Pathogens. *Annu. Rev. Phytopathol.* **46**, 385–418 (2008).
11. Cunniffe, N. J., Cobb, R. C., Meentemeyer, R. K., Rizzo, D. M. & Gilligan, C. A. Modeling when, where, and how to manage a forest epidemic, motivated by sudden oak death in California. *Proc. Natl. Acad. Sci.* **113**, 5640–5645 (2016).
 12. Zhan, J., Ericson, L., González-Jiménez, J. & Burdon, J. J. Disease influences host population growth rates in a natural wild plant–pathogen association over a 30-year period. *J. Ecol.* **110**, 173–184 (2022).
 13. Susi, H., Thrall, P. H., Barrett, L. G. & Burdon, J. J. Local demographic and epidemiological patterns in the *Linum marginale*–*Melampsora lini* association: a multi-year study. *J. Ecol.* **105**, 1399–1412 (2017).
 14. Gehman, A.-L. M., Hall, R. J. & Byers, J. E. Host and parasite thermal ecology jointly determine the effect of climate warming on epidemic dynamics. *Proc. Natl. Acad. Sci.* **115**, 744–749 (2018).
 15. Kim, K.-H., Cho, J., Lee, Y. H. & Lee, W.-S. Predicting potential epidemics of rice leaf blast and sheath blight in South Korea under the RCP 4.5 and RCP 8.5 climate change scenarios using a rice disease epidemiology model, EPIRICE. *Agric. For. Meteorol.* **203**, 191–207 (2015).
 16. Lawrence, G. J., Dodds, P. N. & Ellis, J. G. Rust of flax and linseed caused by *Melampsora lini*. *Mol. Plant Pathol.* **8**, 349–364 (2007).
 17. Moss, R. H. *et al.* The next generation of scenarios for climate change research and assessment. *Nature* **463**, 747–756 (2010).
 18. Wood, S. N. *Generalized additive models: an introduction with R.* (Chapman and Hall/CRC, 2006).

19. Hart, H. Factors affecting the development of Flax rust, *Melampsora lini* (Pers.) Lev. *Phytopathology* **16**, 185–205 (1926).
20. Okubo, A. & Levin, S. A. A Theoretical Framework for Data Analysis of Wind Dispersal of Seeds and Pollen. *Ecology* **70**, 329–338 (1989).
21. Pegg, G. *et al.* Impact of *Austropuccinia psidii* (myrtle rust) on Myrtaceae-rich wet sclerophyll forests in south east Queensland. *PLoS One* **12**, e0188058–e0188058 (2017).
22. Beresford, R. M., Shuey, L. S. & Pegg, G. S. Symptom development and latent period of *Austropuccinia psidii* (myrtle rust) in relation to host species, temperature, and ontogenic resistance. *Plant Pathol.* **69**, 484–494 (2020).
23. Flory, S. L. & Clay, K. Pathogen accumulation and long-term dynamics of plant invasions. *J. Ecol.* **101**, 607–613 (2013).
24. Stricker, K. B., Harmon, P. F., Goss, E. M., Clay, K. & Luke Flory, S. Emergence and accumulation of novel pathogens suppress an invasive species. *Ecol. Lett.* **19**, 469–477 (2016).
25. Grulke, N. E. The nexus of host and pathogen phenology: understanding the disease triangle with climate change. *New Phytol.* **189**, 8–11 (2011).
26. Cunniffe, N. J. & Gilligan, C. A. Invasion, persistence and control in epidemic models for plant pathogens: the effect of host demography. *J. R. Soc. Interface* **7**, 439–451 (2010).
27. Earn, D. J. D., Rohani, P., Bolker, B. M. & Grenfell, B. T. A Simple Model for Complex Dynamical Transitions in Epidemics. *Science* **287**, 667–670 (2000).
28. Burdon, J. J. & Thrall, P. H. Coevolution of plants and their pathogens in natural habitats. *Science* **324**, 755–756 (2009).
29. Thrall, P. H. *et al.* Rapid genetic change underpins antagonistic coevolution in a natural

- host-pathogen metapopulation. *Ecol. Lett.* **15**, 425–435 (2012).
30. Thrall, P. H. & Burdon, J. J. Evolution of virulence in a plant host-pathogen metapopulation. *Science* **299**, 1735–1737 (2003).
 31. Thrall, P. H. & Burdon, J. J. Effect of resistance variation in a natural plant host-pathogen metapopulation on disease dynamics. *Plant Pathol.* **49**, 767–773 (2000).
 32. Antonovics, J., Thrall, P. H., Burdon, J. J. & Laine, A. L. Partial resistance in the linum-melampsora host-pathogen system: Does partial resistance make the red queen run slower? *Evolution (N. Y.)*. **65**, 512–522 (2011).
 33. Kittel, T., Thornton, P. E., Royle, J. andrew & Chase, T. Climates of the Rocky Mountains: Historical and Future Patterns. in 59–92 (2002).
 34. Johnston, B. C., Huckaby, L., Hughes, T. J. & Pecor, J. *Ecological types of the Upper Gunnison Basin: Vegetation-soil-landform-geology-climate-water land classes for natural resource management. Technical Report R2-RR-2001-01.*
https://www.fs.usda.gov/Internet/FSE_DOCUMENTS/fsbdev7_002599.pdf (2001).
 35. Vano, J. *et al.* Comparing Downscaled LOCA and BCSD CMIP5 Climate and Hydrology Projections-Release of Downscaled LOCA CMIP5 Hydrology. *Livermore, CA Lawrence Livermore Natl. Labs* (2020).
 36. Pierce, D. W., Cayan, D. R., Maurer, E. P., Abatzoglou, J. T. & Hegewisch, K. C. Improved Bias Correction Techniques for Hydrological Simulations of Climate Change. *J. Hydrometeorol.* **16**, 2421–2442 (2015).
 37. Pierce, D. W., Cayan, D. R. & Thrasher, B. L. Statistical Downscaling Using Localized Constructed Analogs (LOCA). *J. Hydrometeorol.* **15**, 2558–2585 (2014).
 38. Maurer, E. P., Brekke, L., Pruitt, T. & Duffy, P. B. Fine-resolution climate projections

- enhance regional climate change impact studies. *Eos, Trans. Am. Geophys. Union* **88**, 504 (2007).
39. Hurrell, J., Visbeck, M. & Pirani, P. WCRP coupled model intercomparison project-phase 5-CMIP5. *Clivar Exch.* **16**, 1–52 (2011).
 40. Meehl, G. A. *et al.* Climate Change Projections in CESM1(CAM5) Compared to CCSM4. *J. Clim.* **26**, 6287–6308 (2013).
 41. Littlefield, L. J. & Bracker, C. E. Ultrastructure and development of urediospore ornamentation in *Melampsora lini*. *Can. J. Bot.* **49**, 2067–2073 (1971).
 42. Wood, S. N. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *J. R. Stat. Soc. Ser. B (Statistical Methodol.* **73**, 3–36 (2011).
 43. Marra, G. & Wood, S. N. Practical variable selection for generalized additive models. *Comput. Stat. Data Anal.* **55**, 2372–2387 (2011).
 44. Salas-Eljatib, C. & Weiskittel, A. R. On studying the patterns of individual-based tree mortality in natural forests: A modelling analysis. *For. Ecol. Manage.* **475**, 118369 (2020).
 45. Bolton, D. The Computation of Equivalent Potential Temperature. *Mon. Weather Rev.* **108**, 1046–1053 (1980).

Conclusion

Overview

Infectious diseases will remain dynamic threats in changing world marked by climate change, globalization, and many other factors that shape the potential for pathogens to spread and evolve. The work presented in this dissertation advances knowledge for anticipating (Chapters II and IV) and mitigating (Chapters I and III) these threats. However, numerous challenges remain. There are many questions left to investigate, but also many opportunities to implement current knowledge. I conclude by discussing how my findings relate to future directions for two broad categories of inquiry and action. First, I consider the role of vaccines in mitigating the burden of infectious disease and argue that current knowledge should prompt a shift towards prioritizing transmission suppression. Next, I turn to the field of plant pathology and discuss how a population-level epidemiological perspective could fill key knowledge gaps and uncover opportunities for better interventions.

Vaccines

The primary goal of vaccination is to limit the death and disease caused by pathogens. Vaccines are currently regulated as purely medical tools, and as such, their efficacy is determined based upon their direct effects on the individuals who receive them. From this point of view, there are two non-exclusive mechanisms through which vaccines can achieve their desired effect: *i*) by preventing or reducing the odds of infection and *ii*) by lessening the severity of infection if it occurs (this framework can be viewed as a simplification of the models of vaccine action

presented in chapters I and III). If considering the effects of a vaccine in a vacuum, there is no reason why either mechanism *i*, *ii*, or any combination of *i* and *ii* should be preferred, as long as the overall expected reduction in disease severity for vaccinated individuals is the same. This conclusion changes if epidemiological and evolutionary considerations are taken into account.

The epidemiological benefits of infection-reducing vaccinal immunity (*i*), including a reduction in the force of infection and the potential for pathogen eradication, are widely recognized and appreciated [1,2]. This dissertation (chapters I and III) and the work it builds upon [3] illustrate that infection-reducing effects of vaccines are vital for limiting the potential for the evolution of increased quantitative virulence (i.e. greater rates of damage or mortality). Infection-reducing effects are also crucial for limiting pathogen qualitative virulence evolution (i.e. the ability to evade host defenses and establish an infection), as reductions in transmission reduce the potential for pathogen evolution [4]. Pathogen evolution has traditionally been considered a ‘second order problem’ to be considered only after the immediate threat posed by a pathogen is contained. However, the repeated emergence of SARS-CoV-2 variants [5,6] suggests that this paradigm warrants reconsideration, at least in the context of qualitative virulence evolution.

Infection-reducing vaccine effects have clear benefits for limiting the spread and evolution of pathogens, and in theory, can achieve the same overall expected reductions in disease severity as severity-limiting vaccine effects. As such, processes for vaccine evaluation should be updated to prioritize transmission limiting effects while maintaining current standards for personal protection. Intranasal vaccines that induce a mucosal IgA response could provide this desired infection-reducing effect [7]. Such vaccines could be used alone, or in conjunction with intramuscular vaccines. Concurrent intranasal and intramuscular delivery could potentially

provide robust protection against infection and disease. Alternatively, intranasal booster doses could maintain protection against infection temporarily afforded by intramuscular vaccines. This is one possibility for bolstering the efficacy of COVID-19 vaccines whose infection reducing effects wane over time [8]. Current successes and setbacks surrounding intranasal COVID-19 vaccines offer a first glimpse at what the future of vaccination might hold [9].

Plant pathology

In chapter IV, I generated predictions about how climate change might affect the epidemiology of flax rust. I took a population-level, mechanistic approach in my analyses. This type of methodological framework is uncommon in the world of plant pathology and offers a significant opportunity to generate further insights about the effects of climate change on the ecology, epidemiology, and evolution of plant pathosystems (Fig. 1). This could be accomplished by 1) identifying specific effects of climate change on host and pathogen ecology (broadly encompassing demography, phenology, immunology, and physiology), 2) incorporating these effects into an epidemiological framework, and 3) extending this epidemiological framework to an evolutionary scale using an adaptive dynamics approach similar to the one taken in chapters I and III. As demonstrated in chapter IV, the flax rust model system is an excellent empirical tool for both parameterizing models and testing predictions. Most (if not all) of the ecological and epidemiological factors in Fig. 1 can be easily measured or observed in this field system, and both qualitative and quantitative virulence and resistance traits are known to be present.

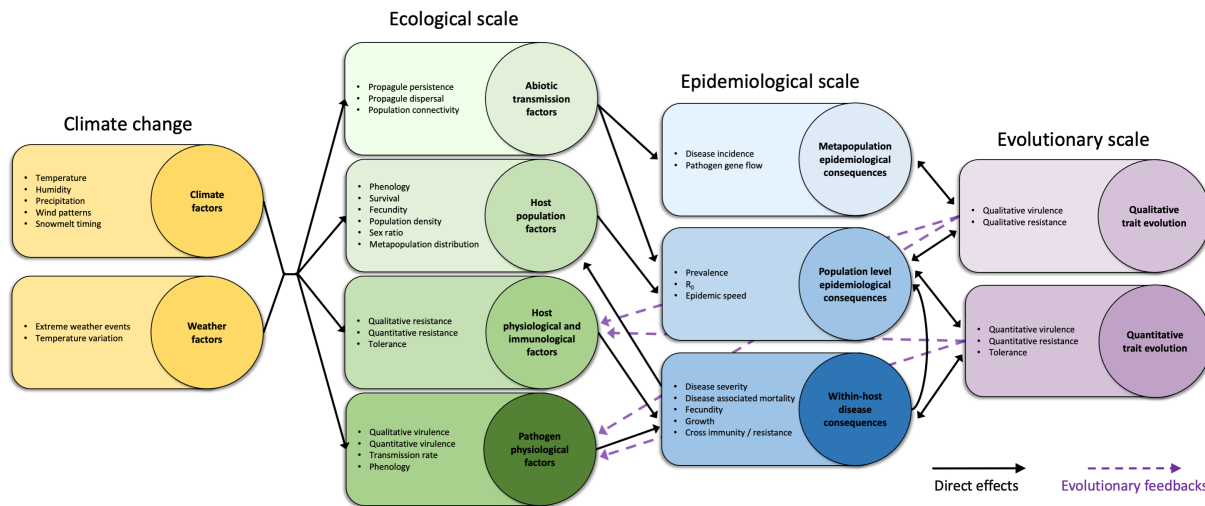


Figure 1: A holistic mechanistic framework for the effects of climate change on the ecology, epidemiology, and evolution of plant pathosystems

Figure 1 illustrates the diverse set of processes that could be linked to climate change and predicted via a mechanistic modeling approach. Targeted models encompassing a small subset of these processes will likely be the most useful for generating predictions.

A mechanistic approach centered on population-level epidemiology could also yield insights pertinent to the management of disease in agricultural systems. In most widely cultivated crops, management practices are focused on qualitative resistance, and involve deploying “R genes” that completely prevent infection. Pathogen evolution poses a significant challenge as it can rapidly erode this engineered protection. The importance of engineering evolutionary durable R-gene resistance is increasingly recognized [10]. Management practices focused on slowing the spread of infection by deploying lines that display quantitative resistance are uncommon, as this practice is believed to select for increased quantitative virulence [10,11].

Qualitative and quantitative virulence traits are known to be inversely correlated in several plant pathosystems [12,13]. This relationship points to the potential for quantitative

resistance to decrease the fitness of an R-gene-evading pathogen line by further dampening its transmission rate. Such strategies for deploying R-genes and quantitative resistance together could be evaluated through the field studies of flax rust and mechanistic modeling. This approach also holds opportunity for unravelling the processes through which quantitative and qualitative virulence and resistance traits co-evolve over time following a novel host-pathogen association [14].

References

1. Anderson RM, May RM. 1985 Vaccination and herd immunity to infectious diseases. *Nature* 318, 323–329. (doi:10.1038/318323a0)
2. Ashby B, Best A. 2021 Herd immunity. *Curr. Biol.* 31, R174–R177. (doi:https://doi.org/10.1016/j.cub.2021.01.006)
3. Gandon S, Mackinnon MJ, Nee S, Read AF. 2001 Imperfect vaccines and the evolution of pathogen virulence. *Nature* 414, 751–756. (doi:10.1038/414751a)
4. Wen FT, Malani A, Cobey S. 2021 The Potential Beneficial Effects of Vaccination on Antigenically Evolving Pathogens. *Am. Nat.* 199, 223–237. (doi:10.1086/717410)
5. del Rio C, Malani PN, Omer SB. 2021 Confronting the Delta Variant of SARS-CoV-2, Summer 2021. *JAMA* 326, 1001–1002. (doi:10.1001/jama.2021.14811)
6. Karim SSA, Karim QA. 2021 Omicron SARS-CoV-2 variant: a new chapter in the COVID-19 pandemic. *Lancet* 398, 2126–2128. (doi:10.1016/S0140-6736(21)02758-6)
7. van Riet E, Aina A, Suzuki T, Hasegawa H. 2012 Mucosal IgA responses in influenza virus infections; thoughts for vaccine design. *Vaccine* 30, 5893–5900. (doi:https://doi.org/10.1016/j.vaccine.2012.04.109)

8. Goldberg Y et al. 2021 Waning Immunity after the BNT162b2 Vaccine in Israel. *N. Engl. J. Med.* 385, e85. (doi:10.1056/NEJMoa2114228)
9. Rubin R. 2021 Trying to Block SARS-CoV-2 Transmission With Intranasal Vaccines. *JAMA* 326, 1661–1663. (doi:10.1001/jama.2021.18143)
10. Zhan J, Thrall PH, Papaix J, Xie L, Burdon JJ. 2015 Playing on a Pathogen’s Weakness: Using Evolution to Guide Sustainable Plant Disease Control Strategies. *Annu. Rev. Phytopathol.* 53, 19–43. (doi:10.1146/annurev-phyto-080614-120040)
11. Delmotte F, Mestre P, Schneider C, Kassemeyer HH, Kozma P, Richart-Cervera S, Rouxel M, Delière L. 2014 Rapid and multiregional adaptation to host partial resistance in a plant pathogenic oomycete: Evidence from European populations of *Plasmopara viticola*, the causal agent of grapevine downy mildew. *Infect. Genet. Evol.* 27, 500–508. (doi:10.1016/j.meegid.2013.10.017)
12. Bruns E, Carson ML, May G. 2014 The jack of all trades is master of none: A pathogen’s ability to infect a greater number of host genotypes comes at a cost of delayed reproduction. *Evolution (N. Y.)*. 68, 2453–2466. (doi:10.1111/evo.12461)
13. Thrall PH, Burdon JJ. 2003 Evolution of virulence in a plant host-pathogen metapopulation. *Science* 299, 1735–1737. (doi:10.1126/science.1080070)
14. Miller IF, Metcalf CJE. 2019 Evolving resistance to pathogens. *Science* 363, 1277–1278. (doi:10.1126/science.aaw8710)

Acknowledgements

There are many, many people to whom I am thankful for support, encouragement, mentorship, and friendship over the course of my PhD. Because of them, I am in the happy and lucky position of finishing my graduate education having accomplished more than I thought was possible. Adequately acknowledging all the help I have received would require another dissertation-length document, but I will try my best to briefly express my thanks here.

I'll begin by thanking my financial supporters. I am immensely grateful for funding from a National Science Graduate Research Fellowship and the Princeton Centennial Fellowship. Without research grants from the department of Ecology and Evolutionary Biology, the Princeton High Meadows Institute Wallbridge Water Grand Challenges funds, the Princeton Center for Health and Wellbeing, and the Rocky Mountain Biological Laboratory (RMBL), my research in the flax rust system would not have been possible.

I am very appreciative of the people who have provided logistical and organizational support for my education and research. Sandy Cominski, Ksenia Rodionova, Lolly O'Brien, Sandra Kaiser, Martha Murdough, Stephen Nappa, Jesse Saunders, Tina Hansen, and many others in the department of Ecology and Evolutionary Biology at Princeton have given me a huge amount of help over the past five years. Emily Ahmetaj and Stacey Christian in the Princeton High Meadows Institute helped me organize funding and logistics for my work with flax rust. At the Rocky Mountain Biological Laboratory, Jennie Reithel, billy barr, Katie Harper, Brett Biebuyck, Kay Petersen Cook, the dining hall crew, the maintenance crew, and the entire staff helped my field research run smoothly.

I am extremely grateful for the mentors who prepared me to pursue a PhD. Emme Bruns and Janis Antonovics helped me to discover the field of infectious disease ecology and evolution. Along with Michael Hood, they also taught me how to study plant pathogens in the mountains. Without them, I would never have pursued science. I learned an immense amount about writing, coding, and critical thinking from Charlie Nunn, who helped to transform me from an overeager undergrad into a scientist ready to take on a PhD.

As a PhD student, I've been exceptionally lucky to continue to be guided by a world class group of mentors. I've had the pleasure of learning from all the faculty in Ecology and Evolutionary Biology at Princeton, including Andy Dobson who was always full of enthusiasm about disease ecology and natural history, and Cassie Stoddard who helped guide me through my first field seasons as the leader of a research team. I learned a lot from working with Lars Hedin and many others during my time as graduate representative. At RMBL, Jennie Reithel and Ian Billick helped me to grow as a scientist and leader, and trusted me to take on the non-traditional role of graduate student principal investigator. I owe my dissertation committee special thanks for their mentorship, guidance, and enthusiasm. Bryan Grenfell, Andrea Graham, and Jonathan Levine all pushed me to ask hard questions and pursue big ideas.

The most important mentor throughout the course of my PhD has been my advisor, Jess Metcalf. I came to Princeton with a crazy plan to pursue questions about human infectious disease epidemiology while also starting a new field research program focused on plant pathogens. Rather than steering me towards something safer and less ambitious, Jess backed me wholeheartedly. She gave me the freedom to take risks, but was always at the ready to provide guidance. Over the course of many fast-paced projects related to the COVID-19 pandemic, she taught me to find useful answers to complicated questions, even when reliable data are scarce. I

learned so much from working with her, and I feel extremely fortunate to have had that experience.

Many other friends and colleagues at Princeton were also important sources of knowledge and encouragement. The Metcalf lab members and the rest of the ‘Disease Group’ were interesting and genuinely enjoyable people to work with. I was inspired by all the other PhD students in my cohort, and we had many good times together, especially during our field ecology trip to South Africa. Jarome Ali was an amazing roommate. Zoe Volenec and Merlijn Staps were the best co-graduate representatives I could have asked for. Alex Becker was a superb mentor, collaborator, and travelling buddy. I’m thankful for the entire EEB graduate student body, which was integral to my experience at Princeton.

My field work with flax rust was one of the most formative and enjoyable parts of my PhD. This has largely been because of the people that I’ve had the opportunity to work with, specifically my field teams. Over the past three years, ‘Team Flax’ has included Juliana Jiranek, McKenna Brownell, Maria Stahl, Barrett Gray, Sarah Coffey, Keenan Duggal, and Andrea Escoto. I am deeply appreciative for the hard work that all of these individuals contributed in the field and through and project development, data analysis, and writing. I am even more grateful for all of the good times, adventures, and friendships that have resulted.

There were many other factors underlying the success of the flax rust system. Bitty Roy provided me with invaluable encouragement when I was first exploring the idea of studying plant pathogens in the Rocky Mountains. During my first exploratory season working with flax rust, the USFS GMUG office helped me secure research permits, and Lauren Carley helped connect me to RMBL. The RMBL community provided a continuous stream of ideas and entertainment. The Crested Butte True Value hardware store and the Gunnison Walmart reliably provided me

many types of odd field equipment. Enterprise consistently overlooked the egregious amounts of mud splattered all over and inside of my team's rental cars. Butte Bagels, Secret Stash, Pitas in Paradise, and many other Crested Butte establishments helped me and my team connect to the community and replace the many calories that we burned hiking to our field sites. The Maroon, Augusta, and Crystal cabins were luxurious (by field standards) accommodations. Cat and Tom Miller provided me and my wife with a home away from home whenever we needed it during the field season. My Aunt and Uncle, Ann and Bill Iwata, were always looking out for me when I was in the field. Visiting them (and Monterrey House) was the perfect way to start and end each field season. I'm deeply grateful for the mountains and landscapes in which I was lucky enough to work, and for the people and organizations that have preserved them and continue to facilitate access.

Friends and family that have given me a huge amount of support and happiness over the past five years of my PhD and beyond. I really appreciated visits in the field from Connor Andrews, Peter Siebers, Mason Brannon, Matthew Kincaid, Catlin Smith, and Elizabeth Carls. The whole 'Bachelor Mountain' crew has been full of encouragement, and endless antics. Spencer and Carson Pigg have been the best neighbors I could ask for, and Lindsay Grier has been an ideal running buddy.

My brothers Alec and Malcolm always have my back, always make me laugh, and are always up for anything. In addition to helping me out in life, they've also helped a lot with my research. Malcolm was patient enough to teach me the basics of coding in C, and once I somehow managed to rope Alec in to helping me collect data at night in sub-freezing temperatures. My parents (Amy and Pete) have always believed in me and encouraged me to take on big challenges and to venture outside my comfort zone. I deeply appreciate the help that

my Aunt Ann and Uncle Bill have given me throughout all my education. My entire extended family has been extremely supportive, and I can't thank them enough.

Lastly, I would like to thank my wife, Emily, who has been a constant source of happiness, comfort, and encouragement. She supported me through all the challenges that I've experienced over the past five years of my PhD, and helped me to keep things in perspective and celebrate accomplishments. While spending the summers at RMBL as an organizational consultant for the field station, she also played an important role on my field teams by appointing herself 'fun captain'. I am incredibly thankful for her, and all that she has done to for me.