

Selecting hyperparasites for biocontrol of Dutch elm disease

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Hyperparasites in the form of cytoplasmic RNA elements have been proposed as a biological control agent for Dutch elm disease. We characterized the range of outcomes likely to follow the introduction of such an agent by modelling the resultant population dynamics as an ecological interaction between the wild 'target' fungus and the hyperparasitized 'control' fungus. We used data from the 1970s epidemic of Dutch elm disease in the UK to parameterize the population dynamics of the target fungus, and considered the success of control across a wide range of possibilities for the lethality and transmissibility of the modified control fungus. We decomposed hyperparasite transmissibility into horizontal transmissibility (the ability to colonize previously unparasitized target fungal hosts) and vertical transmissibility (the ability of control fungus to establish new colonies). There is an invasion threshold for both horizontal and vertical transmissibility. As vertical transmission is further increased, there is another threshold at which the target fungus is eradicated because of competitive exclusion by the control fungus. In contrast, eradication by raising horizontal transmission may never succeed because the target fungus needs to be present to support new cases through this route. Between these two thresholds for invasion and exclusion, the control and target fungi may coexist. Using a stochastic, spatially extended model, we showed that predictions of success based on high competitive ability of the control fungus (i.e. high vertical transmission) are likely to be more robust than those based on the high degree to which the control fungus can cause the target fungus to be hyperparasitized (i.e. high horizontal transmission).

Keywords: biological control; Dutch elm disease; stochastic model; hyperparasite; transmissible hypovirulence

1. INTRODUCTION

There is no effective means of control for Dutch elm disease (Gibbs *et al.* 1994). One attractive possibility is the use of naturally occurring 'd-factors', typically consisting of mitochondrial-associated RNA (Brasier 1983, 1986a; Rogers *et al.* 1986; Hong *et al.* 1998), to infect the causal fungus (*Ophiostoma novo-ulmi* Brasier) and, thus, reduce the impact of the fungus on the host elm population (Buck 1988). A particularly appealing feature of this approach is that the d-factor is naturally transmissible to other fungal colonies. This gives rise to the idea of 'transmissible hypovirulence' and the notion that it might be possible to control infection in large forest stands without extended direct intervention (Brasier 1990; Buck 1988).

Though there are hopes of experimental releases soon, the d-factor remains only a putative control agent at present (Brasier 1996b), and a number of key problems concerning the genetics and epidemiology of transmission have to be resolved if it is to be successful (Buck 1988). One major issue is the loss of d-factors during sexual reproduction (Brasier 1986b). Another is the vegetative compatibility (VC) system of the fungus whereby the

transmission of d-factors through hyphal anastomosis can occur only between fungi of compatible VC groups, so that VC diversity can provide a major barrier to the spread of d-factors in a population (although studies on the analogous h-factors, which hyperparasitize the chestnut blight fungus, suggest that the VC diversity there does not correlate completely with h-factor transmission; Causin *et al.* 1995). Nevertheless, hopes have been expressed that d-factors might be useful biological control agents (Buck 1988), perhaps through incorporation of the d-factor into the fungal genome (Brasier 1996b). In this paper, we address the key epidemiological questions for control by d-factors under the optimistic assumptions that such a transformation can indeed be carried out for Dutch elm disease d-factors or that, in any case, the problems associated with VC compatibility and sexual reproduction are not limiting. In particular we ask the following. Is control guaranteed? What epidemiological properties should be used to select the best d-factors from any available panel (Sutherland & Brasier 1995, 1997) in order to optimize control? Will the d-factor-infected strain replace the indigenous pathogenic strain? Will both persist or will the d-factor die out? How is persistence of the control agent influenced by spatial structure and stochasticity, which are known to be very important in influencing persistence properties in general (Grenfell & Dobson 1995)? We analyse the success of control under population dynamic criteria of invasion,

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coexistence and persistence of the hyperparasitized fungus. Finally, we discuss the consequences for the model of releasing some of the optimistic assumptions about transmission on the dynamics of persistence.

The fungal life cycle is summarized in figure 1, in which we identify two dynamic features, lethality and transmissibility, which govern the success of d-factor control (Webber 1993). Lethality is a measure of the effect that the hyperparasitized fungus has on the elm population, while transmissibility is a measure of the ability of the d-factor to spread through the fungal population. The fungal life cycle (figure 1) is conventionally split into a parasitic and a saprophytic phase. The parasitic phase is initiated by the introduction of the fungus, with or without the d-factor, into the xylem by infested bark beetles feeding on healthy trees (Webber & Brasier 1984). A severe wilt ensues as xylem vessels become blocked and the tree dies. The fungus invades and grows saprophytically within the bark of dead or dying trees, which also provides breeding galleries for the beetle vector. Emerging beetles carry fungal spores to healthy trees. Hence, the parasitic phase yields a supply of dead trees within which inoculum increases saprophytically for transmission to healthy trees via the beetle vector (Webber *et al.* 1987). We define lethality as the force of infection on healthy elms, which is a composite measure of the ability of the fungus to kill trees and the prevalence of inoculum arising from saprophytic multiplication amongst dead trees. The lethality of a d-factor is defined as the lethality of its modified host.

There are two ways in which transmission of a d-factor can occur (figure 1). The first is horizontal transmission when the d-factor enters an established fungal colony. We refer to the wild-type fungus as the target fungus and the fungus carrying d-factor as the control fungus. Horizontal transmission takes place either in a single tree (through either vegetative growth or spore transport; Brasier 1978), or through coming into contact with target fungus in other trees, which requires a vector that is likely to visit fungal-infested sites. The second mode is vertical transmission, with an infected control colony establishing a new daughter colony also carrying the d-factor. Vertical transmission of the d-factor is thus equivalent to transmission of the control fungus. The d-factors also act in exerting 'a degree of natural control during elm bark colonization' (Webber 1993), that is in reducing vertical transmission.

2. A MODEL FOR THE BIOLOGICAL CONTROL OF DUTCH ELM DISEASE

To make these definitions and their implications more precise, we incorporate them into a simple deterministic model and then develop analytic criteria for hyperparasites to invade and completely infect a fungal population. We then discuss how control and target fungi may coexist when neither is capable of excluding the other. Although very useful for such analyses, a continuous deterministic model breaks down in the face of deep post-epidemic troughs at which infection is very low, followed by recovery of the host population (Mollison 1991). Accordingly, a discrete stochastic version of the model is developed (§ 2a) for use in analysing the dynamic beha-

viour of the system and, since the depth of the troughs is altered by spatial subdivision, we then determine the sensitivity of the results to spatial structure (§ 2d).

(a) *Continuous model*

The most recent European pandemic of Dutch elm disease (Gibbs 1978*b*) was largely the result of the spread of *O. novo-ulmi* Brasier. The progress of that infection was most closely monitored in the UK (Gibbs 1978*a*) and, in a previous paper (Swinton & Gilligan 1996), we introduced a mathematical model for the key transmission processes involved that was capable of reproducing the broad features of the epidemic. Here we adapt that model to allow for the existence of a hyperparasite capable of reducing the lethality of its fungal host. Thus, the density of fungal infection in a population is measured in terms of the number (F) of recently dead trees that were colonized by the fungus as opposed to those uncolonized by the fungus (Y). On the basis that young trees, while susceptible to infection, are too small to provide breeding grounds for the beetle vector (Greig 1994), we also included a sapling class W of juvenile trees of this type, subsequently maturing to adult trees (X), leading to a model of the following form:

$$\dot{W} = rX(1 - X/K) - mW, \quad (1)$$

$$\dot{X} = mW - \mu X - \phi FX, \quad (2)$$

$$\dot{Y} = \phi FX + \mu X - \beta FY - \gamma Y, \quad (3)$$

$$\dot{F} = \beta FY - \gamma F. \quad (4)$$

Thus, we made the simple assumptions that non-disease mortality occurs at a constant rate μ and that regeneration occurs only when the removal of an adult has taken place, modelled by a density-dependent per capita growth term $r(1 - X/K)$. Here K is a representation of the carrying capacity of the region. Trees mature at a rate m . Once adult, the individual risk of xylem infection is given by the force of infection ϕFX . Trees die, either due to such infection or to other causes of mortality, and enter the Y class when they are suitable for colonization by beetles and become colonized by spore-carrying beetles at a rate βFY . There is assumed to be no transmission from the xylem to the bark. All dead trees of types Y and F are removed from the system by becoming unsuitable for beetle breeding at a rate γ . Typical parameter values used in this model are included in table 1.

Here, we retain the same model structure but introduce a single extra compartment: the number, G , of recently dead trees colonized by a control fungus. The control fungus is assumed to have qualitatively similar dynamics to those of the target fungus, except that the d-factor is capable of spreading from the control to the target fungus. Counterbalancing the optimistic assumptions that VC diversity and losses through sexual reproduction are negligible, the model does not make a distinction between control fungus carrying genomically integrated d-factor and control fungus cytoplasmically infected by d-factor.

The model equations now have the form

$$\dot{W} = rX(1 - X/K) - mW, \quad (5)$$

Table 1. Definition of (a) model variables and (b) parameters used in the simulations, based on Swinton & Gilligan (1996)

(Stochastic simulations were carried out as described in the text with $\delta t = 0.001$ yr in all the simulations shown, although specimen runs were not significantly changed at smaller values of δt .)

(a) variable	meaning
W	saplings
X	healthy adult elm
Y	dead elm
F	dead elm infected by target fungus
G	dead elm infected by control fungus

(b) parameter	meaning	typical value
r	elm replacement rate	0.05 yr^{-1}
K	carrying capacity	$15 N_0$
m	maturation rate	0.1 yr^{-1}
μ	mortality rate of uninfected elm	0.01 yr^{-1}
ϕ	lethality	$0.25 N_0^{-1}$
$g\phi$	control fungus lethality	varies
β	target fungus transmissibility	$24 \text{ yr}^{-1} N_0^{-1}$
$v\beta$	control fungus transmissibility	varies
h	horizontal transmissibility	varies
γ	bark decay rate	1.0 yr^{-1}
L^2	$L \times L$ spatial grid	1; 25
ϵ	coupling strength for multiple patch model	0.1; 0.001
N_0	population scaling	10^6 elm

$$\dot{X} = mW - \mu X - (\phi F + g\phi G)X, \quad (6)$$

$$\dot{Y} = (\phi F + g\phi G + \mu)X - (\beta F + v\beta G)Y - \gamma Y, \quad (7)$$

$$\dot{F} = \beta F Y - \gamma F - hGF, \quad (8)$$

$$\dot{G} = hGF + v\beta G Y - \gamma G. \quad (9)$$

We have introduced three new parameters: the lethality of the control fungus, written in the form $g\phi$, the transmissibility of the control fungus (or equivalently the vertical transmissibility of the hyperparasite), written in the form $v\beta$ and the ability of the d-factor to colonize target fungus through horizontal transmission, h . Thus g and v are dimensionless parameters representing the relative lethality and relative vertical transmissibility of the control fungus. Horizontal transmission is given by the parameter h , of the same dimensions as the fungal transmissibility β . A flow diagram of this model is shown in figure 1.

This representation of control in terms of three parameters does not imply that d-factors modify these parameters independently: life-cycle constraints will make them each depend on a suite of correlated characters. However, different d-factors may have different trade-offs: the task is to determine how these trade-offs affect the dynamics of Dutch elm disease.

(b) Invasibility and coexistence

To understand the properties of the model it is helpful to introduce a number of basic reproductive ratios, as listed in table 2. The dynamics of the model with no d-factor present are the same as those previously analysed

Table 2. Basic reproductive ratios for four kinds of invasion

($Y^\circ = \mu K(1 - \mu/r)/\gamma$ is the level of recently dead trees in the absence of any infection, while F^* and G^* are the levels of infected trees when there is no control present and when there is no target present, respectively. These are derived by considering the linear stability properties of the relevant equilibrium points of equations (5) to (9).)

reproductive ratio of	when invading a tree population	symbol	value
target fungus	uninfected	R_0	$\beta Y^\circ/\gamma$
control fungus	uninfected	vR_0	$v\beta Y^\circ/\gamma$
control fungus	infected (uncontrolled)	R_d	$v + hF^*/\gamma$
target fungus	infected (controlled)	R_t	$1/v - hG^*/\gamma$

in Swinton & Gilligan (1996). There are two broad possibilities in the absence of control. If the target fungus is transmissible enough, it remains in the population and depresses elm levels, but otherwise it dies out and elms return to their carrying capacity. A measure of this is provided by the reproductive ratio of the fungus, R_0 , which must satisfy $R_0 > 1$ for the fungus to persist.

Analysis of the extended model (table 3) shows that there is an analogous reproductive ratio, R_d , such that if $R_d > 1$ biological control will become established once introduced and a ratio R_t , which determines whether the target fungus can be eradicated subsequent to establishment. In the absence of horizontal transmission ($h = 0$), the control and target fungus cannot coexist at equilibrium: they are competing for the same niche and the target fungus will be driven extinct if it has a lower vertical transmission. This agrees with the analysis of Anderson & May (1986), but coexistence can occur when horizontal transmission is present, as demonstrated in a similar setting by Lipsitch *et al.* (1995) and illustrated in figure 2.

These analyses describe how possible equilibria change with parameters, but the full dynamic structure is more complex, as illustrated in figure 3a. This shows equilibrium outcomes of the model as a function of vertical transmission v when the horizontal transmission h is fixed. Note that we are not supposing that horizontal and vertical transmission are independent parameters, but use this analysis as a device for studying the transition to control. There is, as predicted by figure 2, a transition to the establishment of control near $v = 0.6$. As vertical transmission increases further, an endemic equilibrium becomes established and levels of healthy trees increase, until eventually the entire population comprises control fungus and elm levels recover to close to their initial value.

It is apparent from figure 3 that the bifurcations may be subcritical and the endemic equilibria may in turn lose stability (at saddle-node or Hopf bifurcations). Indeed, figure 3b, with a control that only mildly reduces lethality ($g = 0.8$), while the relative lethality, g , does not alter the threshold for the establishment of control, it does have a dramatic effect on the level of control once established and creates a region where none of the equilibrium points of the system are stable.

The bifurcation diagram of figure 2 suggests that this qualitative picture also depends on h and, indeed, figure 4

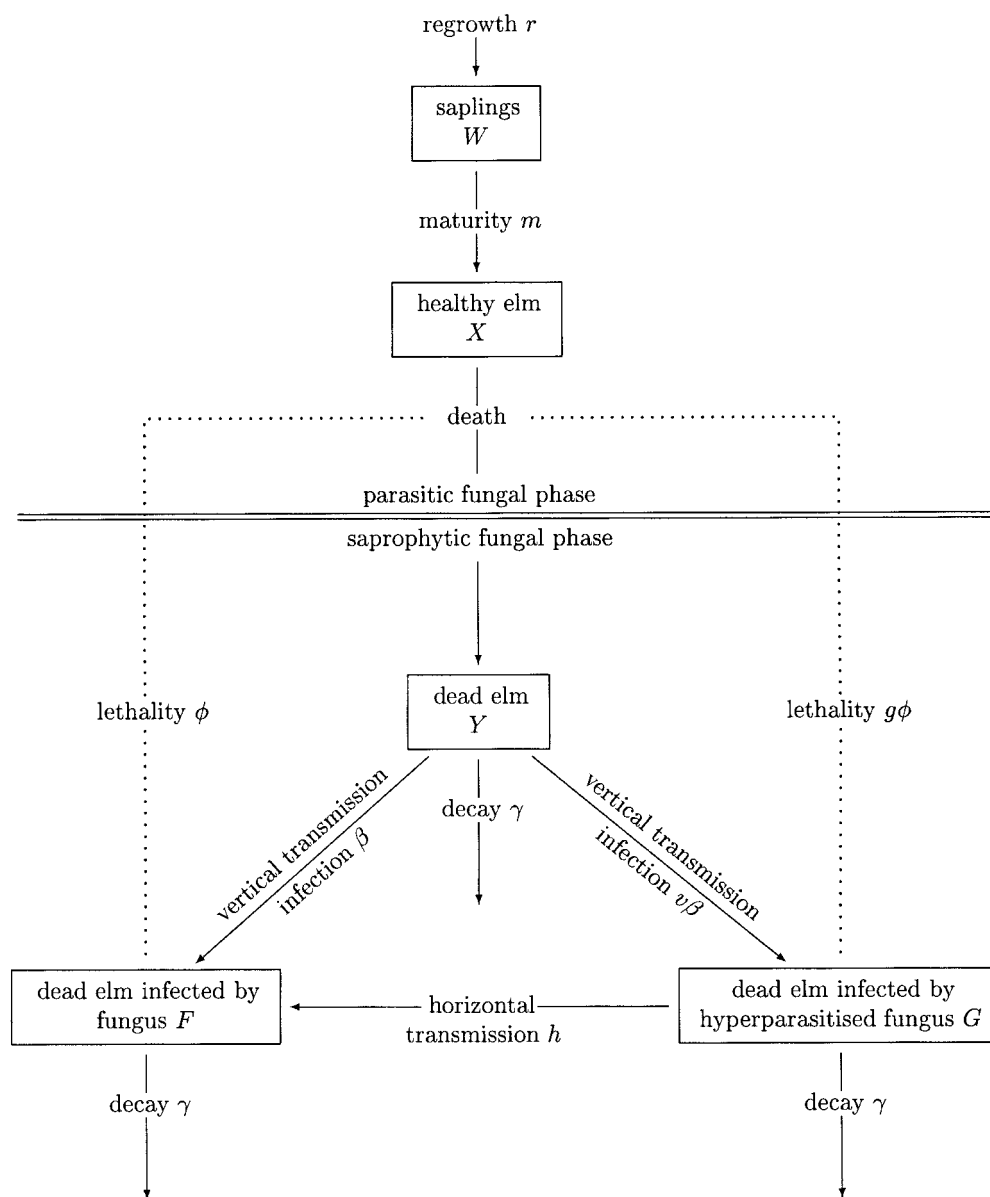


Figure 1. Flow diagram for a Dutch elm disease system in the presence of a hyperparasite, corresponding to the model (5)–(9).

Table 3. Necessary conditions for various outcomes of biological control

equilibrium	(F, G)	requires
disease-free state	$(0, 0)$	$R_0 < 1$ $vR_0 < 1$
control fails	$(F^*, 0)$	$R_0 > 1$ $R_d < 1$
control wins	$(0, G^*)$	$vR_0 > 1$ $R_d > 1$ $R_f < 1$
control persists	(F^e, G^e)	$v < 1$

shows the corresponding diagrams for a range of h values. As h increases, the transition point for the invasion of control becomes easier to reach (i.e. at lower v) until the control can always become established. Thus, it is possible for a very weakly transmissible control fungus ($h \approx 0$) to have a disproportionate effect on elm levels and restore them to near carrying capacity (figure 4*d,e*). This effect is only apparent when the control fungus is not self-sustaining (i.e. $hR_0 < 1$), so that, paradoxically, elm levels

can decline as the relative transmission of the control and less lethal fungus increase.

In summary, a control fungus can outcompete a target one even if the control has a lower lethality. A range of behaviour can be seen in the transition zone, but typically, as the transmissibility increases, the control fungus outcompetes the target. There is an important caveat, however. For much of the parameter range of coexistence, the equilibrium point is unstable, pointing to the importance of considering not only the static but also the dynamic properties of the system, to which we turn in the next section.

(c) **Stochastic non-spatial model**

In order to consider the dynamic behaviour of the biocontrol we now turn to a discrete stochastic equivalent of the deterministic model. We adopt a standard stochastic representation of the epidemic processes (Bailey 1975) in which the number of trees in each class is now taken to be an integer and each of the transitions

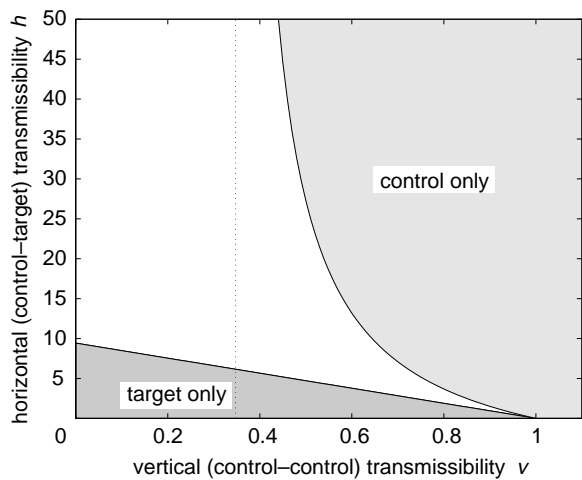


Figure 2. Resistance to invasion as a function of horizontal and vertical transmission. Lower shaded area: target fungus resists invasion of control agent (defined by $R_d < 1$ in table 2). Upper shaded area: control fungus resists invasion of target fungus (defined by $R_t < 1$ in table 2). Parameter values from table 1 with $g = 0$. When the control fungus is transmissible enough (always for $v > 1$, but also $v < 1$ if h is large enough), it is capable of driving the target to extinction; if it is not transmissible enough, the control fungus is driven to extinction by the target fungus and the d-factor is lost; at intermediate levels of transmission both may coexist. The equilibrium at which all fungus is control only exists for $vR_0 > 1$, indicated as a vertical dotted line.

from one class to another is governed by a stochastic process. Thus, for example, the number of juvenile trees is an integer-valued random variable W . Corresponding to the birth $rX(1 - X/K)$ process in the differential equation for dW/dt , we have the stochastic process causing the transition $W \rightarrow W + 1$ with probability $r(1 - X/K)\delta t$ for each X in a small time-step δt . The number of such transitions drawn from a binomial distribution is $B(n, p)$ with $n = X$ and $p = r(1 - X/K)$, but if $np(1 - p) > 25$ or $(np(1 - p) > 5$ and $0.1 \leq p \leq 0.9)$ or $(np > 10$ and $n(1 - p) > 10)$ an approximating normal variate with mean np and variance $np(1 - p)$ is used, while if $p < 0.1$ and $np < 10$ a Poisson variate of mean np is used (Evans *et al.* 1993). Each transition process is modelled in this way.

Figure 5 shows the mean and interquartile range of 100 realizations of the stochastic model and compares them with the corresponding deterministic model. For low levels of horizontal transmissibility, h , (figure 5a,b), the stochastic model follows the deterministic in predicting a transition from non-invasibility to complete control as v is increased. The stochastic model also shows that invasibility is more difficult than deterministically expected near the threshold (Bailey 1975). As horizontal transmission increases down the figure, the depths of the post-epidemic troughs deepen and new phenomena emerge. In figure 5d, control can only persist if it is vertically transmissible enough in its own right (i.e. $vR_0 > 1$) although the deterministic theory predicts that

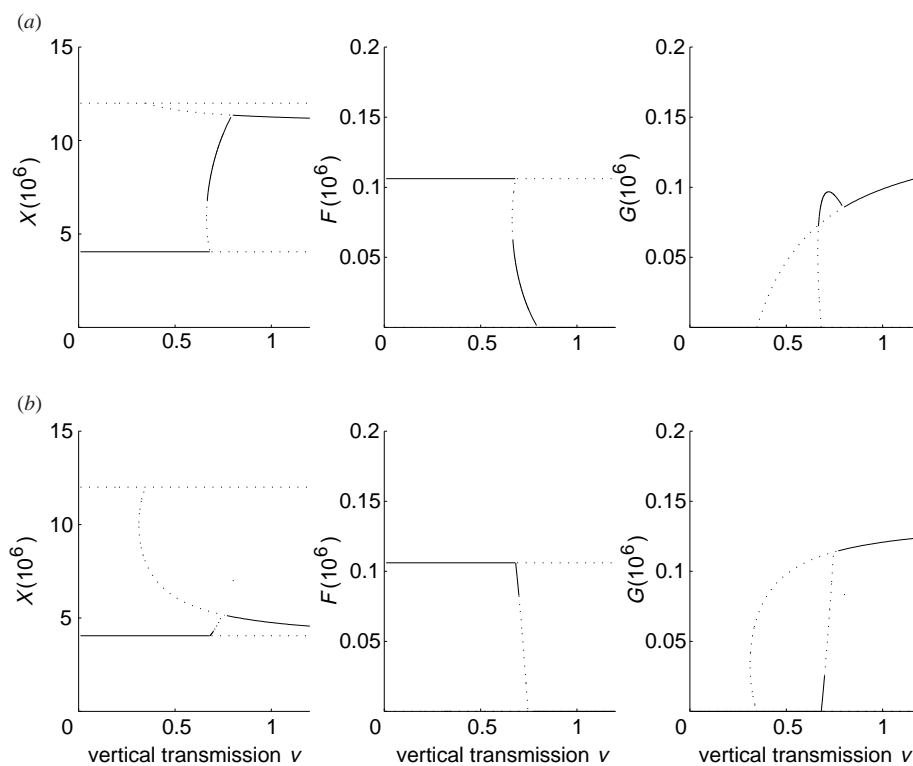


Figure 3. Transition to control as vertical transmissibility increases. Equilibrium levels of healthy elm (left-hand column) and fungal intensities (centre column, target fungus; right-hand column, control fungus) predicted as a function of relative lethality, g , and vertical transmissibility of control, v . Solid lines reflect stable equilibria, which would persist over time; dotted lines indicate unstable equilibria at which the system will not persist after a small perturbation, such as the introduction of a small amount of infection. The results are derived by solution of quadratic equations resulting from setting equations (5)–(9) to zero, followed by numerical evaluation of linear stability conditions. Parameter values as in table 1 with $h = 3.0$ and (a) low lethality ($g = 0.1$) and (b) high lethality ($g = 0.8$).

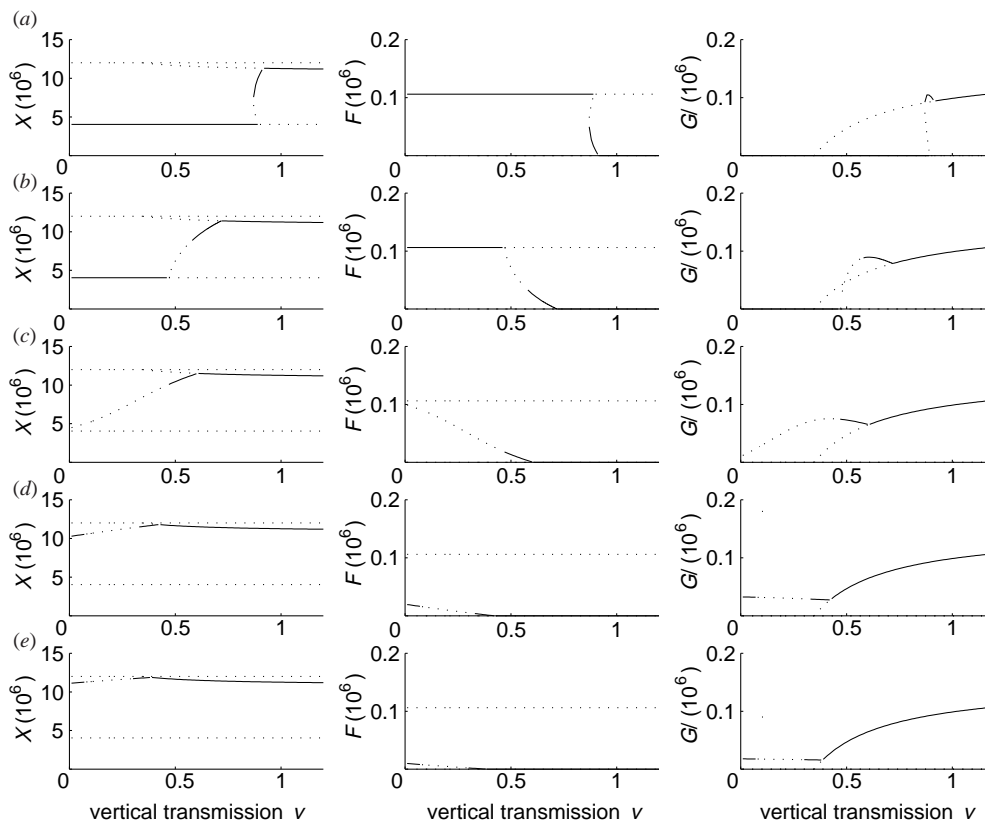


Figure 4. Level of control also depends on horizontal transmission. Equilibrium levels of healthy elm (left-hand column) and fungal intensities (centre column, target fungus; right-hand column, control fungus) predicted as a function of vertical transmissibility, v , and horizontal transmissibility of control, h . Derivation and parameter values as in figure 3 except $g = 0.1$ and $h = (a) 1, (b) 5, (c) 10, (d) 50$ and $(e) 100$.

control fungus can always become established. Although less vertically transmissible agents can invade, high horizontal transmission drives them to extinction in the following trough. In complete contrast, in figure 5e, the control fungus is initially so successful that it colonizes the entire target population. However, in this resulting population, the reduced vertical transmission is too low to allow the control fungus to persist. Thus, the introduction of the control agent leads first to the eradication of the target fungus, followed by the removal of the control agent itself. The advantage of this strategy is outweighed by its risk of failure: since it relies on the interaction between the epidemic dynamics and the intrinsic stochasticity of the system, it is particularly sensitive to perturbations to the system structure. To illustrate this sensitivity, we introduce a spatial structure into the stochastic model.

(d) Stochastic spatial model

We introduce a spatial structure into the stochastic model by considering a number of linked ‘patches’ within the elm population in which the force of infection is a weighted mean of the levels of infection in a patch and the mean in the local neighbourhood, with the relative weight of each term determining the coupling strength. Thus, the deterministic model becomes

$$\begin{aligned} \dot{W}_i &= rX_i(1 - X_i/K) - mW_i, \\ \dot{X}_i &= mW_i - \mu X_i - (\phi\hat{F}_i + g\phi\hat{G}_i)X_i, \\ \dot{Y}_i &= (\phi\hat{F}_i + g\phi\hat{G}_i + \mu)X_i - (\beta\hat{F}_i + v\beta\hat{G}_i)Y_i - \gamma Y_i, \\ \dot{F}_i &= \beta\hat{F}_i Y_i - \gamma F_i - h\hat{G}_i F_i, \\ \dot{G}_i &= h\hat{G}_i F_i + v\beta\hat{G}_i Y_i - \gamma G_i, \end{aligned}$$

with i indexing the patch and the caret denoting a weighted mean of patch and neighbourhood infection: specifically $\hat{F}_i = (1 - \epsilon)F_i + \epsilon\bar{F}_i$ with \bar{F}_i the mean over the four immediate neighbours of patch i with zero contribution from patches outside the grid. All the simulations use a stochastic version of this model as defined in the previous section and a square 5×5 grid.

Figures 6 and 7 show the effect of relatively strong and relatively weak spatial coupling, respectively. When the coupling is strong, the model predicts very similar results to those of the non-spatial model. However, at weak coupling, the spatial structure dramatically reduces the possibility of mutual coextinction (figure 7e). Although the control fungus can drive out the target in each patch, it cannot do so simultaneously in a large number of patches slightly out of phase unless it too can persist in each of those patches. Since vertical transmissibility is more important than horizontal transmissibility in promoting exclusion, predictions of success based on high vertical transmission are likely to be more robust than those based on high horizontal transmission.

However, it is precisely those results sensitive to the stochastic dynamic effects that are modified by additional spatial structure. For $h = 50$, the ability of the target fungus to drive out the control during the post-epidemic trough is weakened and there is more coexistence than in the non-spatial model.

3. DISCUSSION

We have distinguished three components of ‘transmissible hypovirulence’ of fungi: vertical transmissibility, horizontal transmissibility and lethality of the fungus. In general, these are far from independent characteristics of

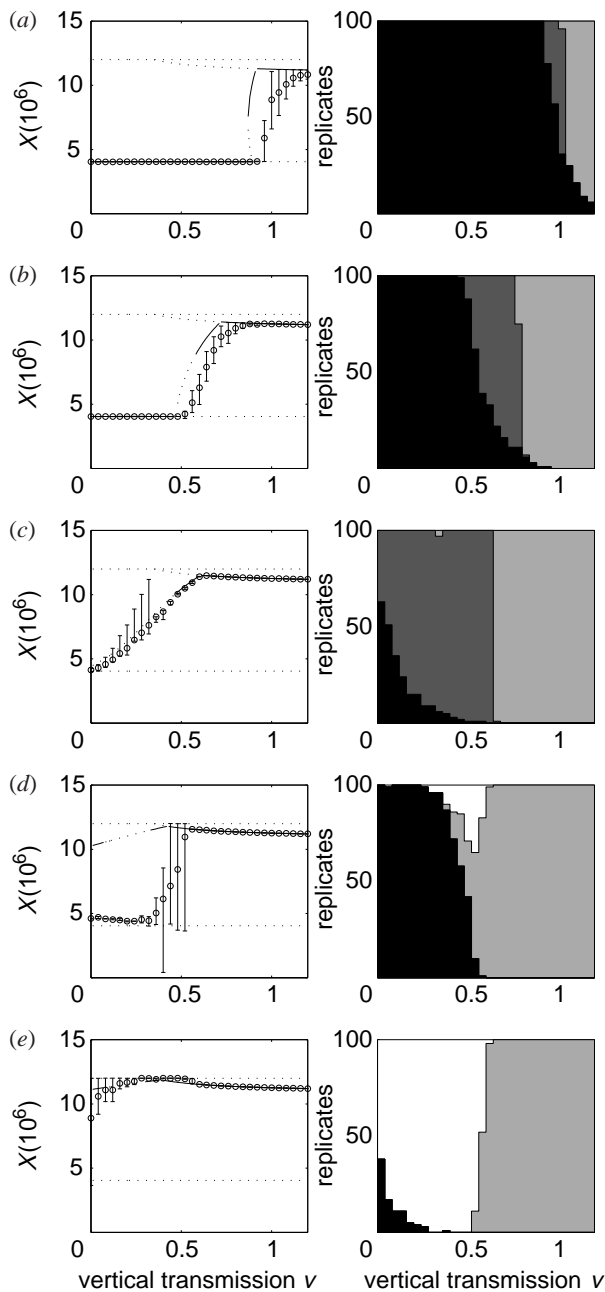


Figure 5. Controlled levels and probability of establishment of control as a function of vertical and horizontal transmission. h values are the same as in figure 3. Left-hand column (healthy elm): elm levels predicted by deterministic model as in figure 4 (solid and dashed lines), together with mean and interquartile range of 100 replicates of the analogous stochastic single-patch model with the same parameters, sampled 200 years after the introduction of control. Right-hand column (coexistence): fraction of replicates in which only target fungus persists (black), both target and control fungi persist (dark grey), only control fungus persists (grey) and neither persists (white). When horizontal transmission is low (h small), the target fungus dominates and elm levels are substantially depressed even when the control fungus is nearly as good a competitor (v near one). As v increases through a critical value of around one, the control fungus becomes able to invade and, since it induces less lethality, elm levels recover. As h is increased, this transition occurs at lower levels of the relative transmission v . For large enough h values, this transition occurs for all v values.

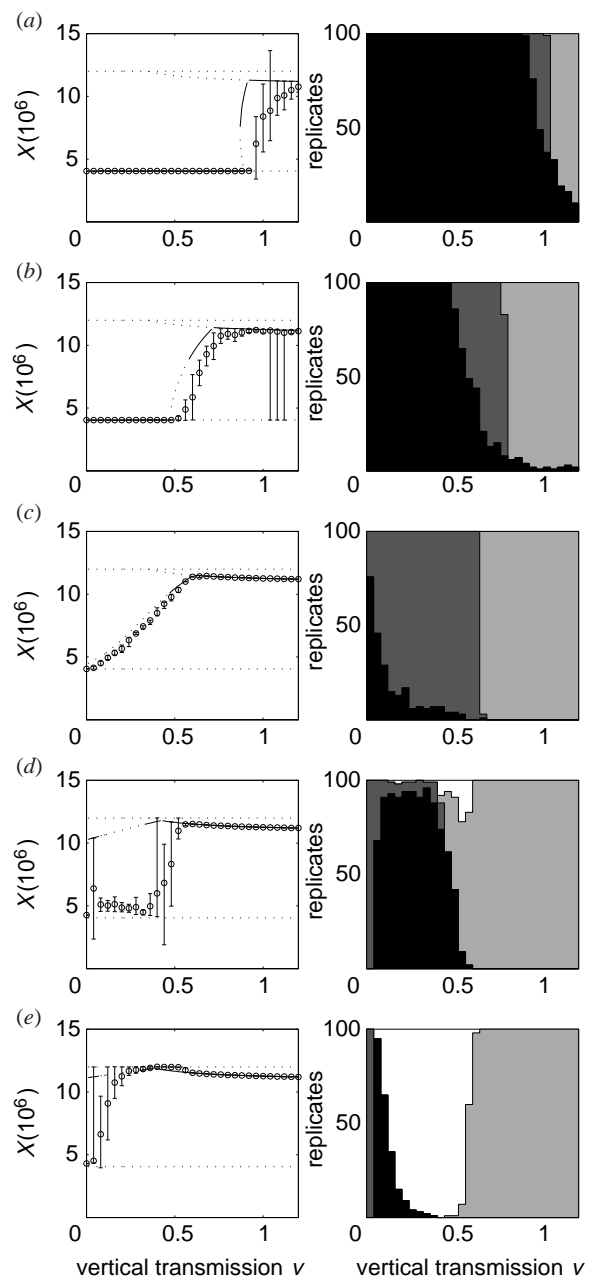


Figure 6. Multiple patches with strong spatial coupling are similar to a single patch. For legend, see figure 5. Strong coupling $\epsilon = 0.1$.

putative d-factors, but one plausible choice to be made might be the relative ability for horizontal (h) or vertical (v) transmissibility. This paper proposes that the most important ability to aim for in this trade-off is a high vertical transmissibility (i.e. high v) and that the primary reason for this is predictability: this simple ability to outcompete and exclude the target fungus is not sensitive to the detailed dynamic behaviour of the system. This is particularly important in the light of our previous observations (Swinton & Gilligan 1996) that the long-term ecological interactions cannot be well predicted from our present knowledge. In contrast, control agents that give up some of this competitive advantage as the price for improved horizontal transmission might still be successful, but the dynamics of the resulting multilevel

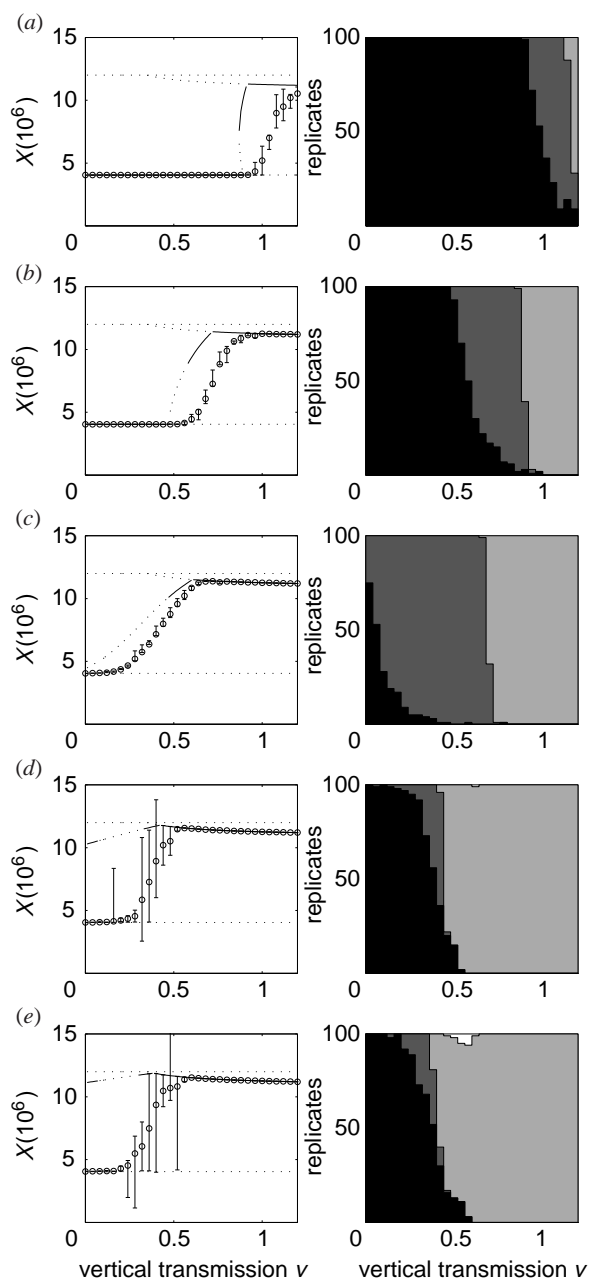


Figure 7. Weak spatial coupling prevents mutual extinction. Bifurcation diagrams for stochastic multipatch model; parameters as in figure 6 but with $\epsilon = 0.001$.

trophic system can be extremely complex. Similar considerations are likely to apply in the choice of biocontrol agents against chestnut blight (Anagnostakis 1995; Taylor *et al.* 1998).

Lethality, in the sense of mortality given transmission, is the commonest subject of experimental studies of transmissible hypovirulence (Ghabrial 1994; Sutherland & Brasier 1995): reducing this is certainly central to the success of control if control can become established, but we have shown that it is the least important of the three components in influencing whether the control can become established.

Other authors (Webber 1993; Sutherland & Brasier 1997) have suggested that the guiding principle should be to select those control agents that are 'moderately deleterious', with the aim of maintaining transmission within the

saprophytic cycle but with reduced ability to infect healthy trees. In the framework of this paper, such a strategy proposes d-factors of low lethality, but recognizes that lethality and transmission are likely to be correlated: Sutherland & Brasier (1997) stated that 'severe' (i.e. low-lethality) d-factors should not be deployed, presumably on the assumption that the same characteristics (causing low growth rates and reduced conidial viability) that make them of low lethality will also make them of low vertical transmission. Rational design of d-factor biocontrol would benefit from empirical studies on the effect of d-factors being extended from the the parasitic to the saprophytic phase to test this assumption and how sensitive it is to the presence of horizontal transmission in particular.

This model has no explicit representation of mating (VC) types and would need to be adjusted if early hopes (Brasier 1996*b*) that genetic modification could overcome this issue were not fulfilled. There is only one known mating type in New Zealand, and in the United States a single type accounts for 60% of isolates with 90% in just three types, although there is evidence from European observations of a proliferation of mating types once an epidemic has been present for five to six years (Brasier 1996*a*).

This paper does not explicitly discuss the coevolution of host, parasite or hyperparasite. It will be useful to extend existing theoretical studies combining ecology and evolution (May 1990; Anderson 1995) to these host-parasite-hyperparasite systems. A substantial start has been made in a paper by Taylor *et al.* (1998), which was published after this paper was submitted, which used a similar continuous deterministic analysis to ours and concluded, among other things, that the most effective biocontrols require high horizontal transmission. Our results are compatible with this conclusion for the deterministic model, but suggest that it might need to be modified in the presence of stochastic dynamic complexity. It would be particularly useful to combine the evolutionary focus of their argument with the complications that we demonstrate follow from the presence of spatial heterogeneity and the possibility of stochastic fade-out.

When considering the mechanism by which a biocontrol agent can become permanent, we have seen that it is important to distinguish horizontal and vertical transmission of the control agent and to be precise about the meaning of lethality or virulence. However, in any analysis of transmissible hypovirulence, the most important distinction to be made is between transmission and virulence.

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