Mathematical model of plant-virus interactions mediated by RNA interference

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HIGHLIGHTS

- Plant virus co-infections.
- RNA interference.
- Viral synergism.

ABSTRACT

Cross-protection, which refers to a process whereby artificially inoculating a plant with a mild strain provides protection against a more aggressive isolate of the virus, is known to be an effective tool of disease control in plants. In this paper we derive and analyse a new mathematical model of the interactions between two competing viruses with particular account for RNA interference. Our results show that co-infection of the host can either increase or decrease the potency of individual infections depending on the levels of cross-protection or cross-enhancement between different viruses. Analytical and numerical bifurcation analyses are employed to investigate the stability of all steady states of the model in order to identify parameter regions where the system exhibits synergistic or antagonistic behaviour between viral strains, as well as different types of host recovery. We show that not only viral attributes but also the propagating component of RNA-interference in plants can play an important role in determining the dynamics.

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1. Introduction

With a projected number of 9.7 billion people by the year 2050, the world population and its continuing growth is heavily dependent on a steady agricultural output in order to provide a sustainable food source. In light of the agricultural stagnation experienced in the last decade, further fuelled by the public opposition to controversial newer practices (Kuntz, 2014), securing an adequate and reliable food source has never been more relevant. It is estimated that up to 40% of global crop production is lost due to pathogens, animals and weeds (Savary et al., 2012). This inevitably led to the development of different agricultural practices including the use of various pesticides and ultimately genetic engineering. Although significant efforts are made to increase crop yield and with a good degree of success, perhaps, a more effective or environmentally safe way to address this problem hides in

better investigating and understanding methods that are currently employed. In this respect, mathematical models can provide invaluable insights into the dynamics of plant infections and allow better control over agricultural losses.

Similar to the studies of infectious disease in humans, mathematical modelling allows one to investigate how an infection propagates within a population of plants. As such, the interactions between healthy and infected plants can usually be described by empirically derived relationships between plants and an insect population which acts as the disease vector and is comparable to epidemic models of mosquito-borne diseases in humans (Purcell and Almeida, 2005). Several mathematical models have also analysed the efficiency of simpler and more traditional methods of fighting plant infection, such as roguing and replanting, in which any plants afflicted by the disease are simply removed and replaced by other healthy plants (Chan and Jeger, 1994; van den Bosch and de Roos, 1996; Zhang et al., 2012).

In the 1970s, the increase of computing power allowed the development of models capable of simulating vector population and weather conditions (Gutierrez et al., 1974; Frazer, 1977; Kirtani

and Sasaba, 1978; Irwin et al., 2000). Despite their simple structure, these models enabled the integration of various disease control options, thus creating a framework where such methods could be analysed and evaluated. Madden et al. (2000) have performed a detailed analysis of the transitional dynamics of plant diseases taking into account the effects of vector emigration. Depending on the way they are transmitted, plant viruses are classified as non-persistent, semi-persistent and persistent, and Madden et al. (2000) demonstrated which of these three classes were more susceptible to changes in vector longevity and inoculation, acquisition rates and vector mobility. Subsequent models have looked into the transmission dynamics of a pair of "helper" and helper-dependent viruses. Zhang et al. (2000) provided insights into the commonly observed phenomenon where infecting a host with only a helper virus would cause minimal or no damage to the host, whereas, additionally introducing the helper-dependent virus would produce far more devastating symptoms.

In the last few decades it has been discovered that viruses employ a wide antigenic diversity as an effective strategy to survive within the host population (Frank, 2002; Lipsitch and O'Hagan, 2007). By employing a variety of antigenic ally distinct strains, viruses are able to adapt sufficiently fast to evade the host's immune system. Antigenic variation is known to be effective for a large number of pathogens affecting humans, including malaria (Gupta et al., 1994; Ferreira et al., 2004), meningitis (Gupta et al., 1996; Gupta and Anderson, 1999), dengue fever (Gog and Grenfell, 2002) and influenza (Ferguson et al., 2003). The interactions between multiple strains are generally classified as either an ecological interference, or an immunological interference. The first type of interactions describes a simple case where individual hosts can only be infected with a single strain, and subsequently the are removed from the population susceptible to other strains (Levin et al., 2004). Immunological interference corresponds to situations where infection with one strain may cause partial or full immunity to the remaining strains (Gupta and Anderson, 1999), or sometimes it can even augment the susceptibility of the host and the transmissibility of other strains (Recker et al., 2009). To better understand the dynamics of multi-strain diseases, a large number of mathematical models have been developed that can be divided into individual-based and equation-based models. In individual-based models, all pathogen strains are treated as individuals interacting according to a fixed set of rules (Ferguson et al., 2003; Buckee et al., 2004; Buckee and Gupta, 2010; Cisternas et al., 2004), whereas in equation-based models, hosts are categorised either according to preceding exposure to individual strains (Andreasen et al., 1997; Gomes et al., 2002), or based on their immunity to specific strains (Gog and Grenfell, 2002; Kryazhimskiy et al., 2007).

One very efficient way of protecting a plant against a disease known as *cross-protection*, consists of the process by which prior infection of the plant with a primary virus can prevent or interfere with the subsequent infection with a secondary virus of the same family (Zhou and Zhou, 2012). In such a case, deliberately infecting the plant with a less virulent strain can offer protection against a much more virulent isolate of the virus. Although this natural phenomenon was first demonstrated more than 80 years ago, its precise mechanisms are still not fully understood, and several hypotheses have been put forward to explain how cross-protection works (Pennazio et al., 2001). It has been suggested that the primary infection could trigger the formation of specific antibodies which could prevent the subsequent infection by a similar virus. Another possibility is the coat-protein mediated resistance that is usually expressed by transgenic plants encoding viral coat-proteins. However, in the case of competing viral strains, the coat protein of the primary strain can also interfere with the

encapsidation process of the secondary strain, thus rendering it ineffective for cell-to-cell transmission (Beachy, 1999; Bendahmane et al., 1997). Additionally, if the two viruses are closely related they could very well be competing for the same components which are essential for viral replication, or that the occupation of replication sites by the primary strain could cause a spatial exclusion of the secondary strain (Lee et al., 2005; Takeshita et al., 2004; Gal-On and Shiboleth, 2006).

A very promising explanation of cross-protection can be found in the biological pathway known as a *post-transcriptional gene silencing*, or *RNA-interference* (RNAi) (Ratcliff et al., 1999). This mechanism is characterised by the ability of cells to recognise and degrade the messenger RNA of invading RNA viruses or cause the methylation of target gene sequences and the genome of DNA viruses (Waterhouse et al., 1999; Escobar and Dandekar, 2003; Sijen and Kooter, 2000). This process is mediated by different lengths of double stranded RNAs (dsRNA) that are generated by an inverted-repeat transgene or an invading virus during its replication process. A very simple description of the core pathway is as follows. The presence of transgenic or viral dsRNA triggers an immune response within the host cell, whereby the foreign RNA is targeted by specialised enzymes called dicers (DLC) which cleave it into short 21–26 nucleotide long molecules. These molecules, named short interfering RNAs (siRNA) or microRNA (miRNA) can then be used to assemble a special protein complex called RNA-induced silencing complex (RISC) which has the capacity to recognise and degrade RNAs containing complementary sequences. By doing so, viral replication is prohibited, and, therefore, it prevents the spread of infection (Costa et al., 2013; Hammond et al., 2000; Bernstein et al., 2001). It is very important to note that the siRNA can also be transported into neighbouring cells, thus acting as a mobile warning signal that can fortify and prepare cells by allowing them to express the antiviral components even before they become infected (Zhang et al., 2012; Wassenegger, 2000; Zhang and Ruvkun, 2012).

The ability to induce a propagating warning signal can most likely be attributed to the evolutionary race between the plant and the viruses that afflict them, as it has been demonstrated that viruses can suppress different stages of the RNA-interference pathway (Costa et al., 2013; Pumplin and Voinnet, 2013; Raja et al., 2008). In some cases the virus can prevent degradation of its genome by either suppressing cellular innate immune response or by simply managing to successfully spread before being detected. The latter can be achieved by moving into another cell before a specific threshold of viral dsRNA has accumulated, and one that is necessary in order for the cell to initiate a response. In other cases, the virus can only suppress the propagating warning signal, therefore, depending on which component of the immune response is targeted by viral suppressors, one can expect a different phenotype of recovery.

It is important to note that in the studies of plant pathology, single-host interactions between different viruses are highly important as they can often produce distinct types of host immune response. Therefore while some viral pairs are able to facilitate each other and engage in a synergistic relationship others will compete with each other for dominance (Malapi-Nelson et al., 2009; Wege and Siegmund, 2007; Pruss et al., 1997). Contrarily to cross-protection, enhanced symptom display occurs when plants co-infected with two or multiple viral strains experience symptoms that are more severe to the single-strain example and often exhibit an elevated viral load for one or multiple viruses. Therefore, depending on the level of competition between the viruses and the corresponding immune response a different degree of cross-protection or cross-enhancement can be observed.

It is most unlikely that any synergistic or antagonistic outcome of a viral co-infection in a single host, associated with cross-

protection or enhanced symptom display, can be fully explained by one single mechanism. This is due to the wide variety of plants with an immune system that is highly specific to the plant, and the fact that different viruses can often produce unique patterns of interactions (Gal-On and Shiboleth, 2006; Roossinck, 2005; Take-shita, 2005; Bergua et al., 2014). However, if one takes different hypotheses into consideration, depending on the sequence homology of the two viruses and their specificity, one of them could inadvertently trigger an immune response or establish a set of host conditions that could either prevent the secondary infection from taking place or allow it to manifest more aggressively (Malapi-Nelson et al., 2009; Chowda Reddy et al., 2012).

Current mathematical models of plant virus epidemics with cross-protection have focussed primarily on the transmission dynamics between populations of healthy plants and plants that are infected with one or multiple viral strains (Zhang et al., 2000; Zhang and Holt, 2001; Jeger et al., 2011). By studying the mechanisms of cross-protection on a cellular level, one might achieve a better understanding of the interactions between two viral strains and a single host. In this paper we derive and analyse a model of a plant disease within a single host with particular account for RNAi-mediated cross-protection. We will show that the model can provide a good qualitative description of the plant's immune response to a viral co-infection, and that it provides a framework in which RNAi can account for both viral synergism and antagonism resulting in cross-protection. A potential application of the model lies in better understanding the efficacy of treating plants against viral diseases by means of the introduction of specific viral strains or genetically modified viruses.

The outline of this paper is as follows. In the next section we describe in detail the main biological assumptions and derive a corresponding mathematical model of plant immune response. In Section 3 we identify all steady states of the model together with conditions for their biological feasibility and stability. Section 4 is devoted to numerical stability analysis of these steady states, as well as numerical simulations of the model to illustrate different types of dynamical behaviour. The paper concludes with the discussion of results and open problems.

2. Model derivation

To investigate the dynamics of biological interactions taking place during a co-infection of a plant with two viruses, we divide the total population of plant cells into the following compartments: healthy (or, susceptible) cells $S(t)$, populations $I_1(t)$ and $I_2(t)$ of cells infectious with virus 1 or virus 2, cells $W_1(t)$ and $W_2(t)$ that are immune to viruses 1 and 2, cells $H_1(t)$ and $H_2(t)$ that have recovered from a primary infection with one of the virus and are currently infectious with the other virus, and finally, the population of super-protected cells $W_{12}(t)$ that are immune to both viruses. Transitions between these different cell populations are illustrated in Fig. 1.

For the sake of model simplicity, spatial components associated with host-specific anatomy will be neglected, and the cell populations are assumed to uniformly distributed within the plant. Despite potentially overlooking some aspects of the dynamics, the assumption of spatial uniformity has been very effectively used to understand viral dynamics (Perelson, 2002; Wodarz et al., 2002). Non-spatial models can provide significant insights into the dynamics and become the basis upon which more detailed models can be built on. Additionally, in the case of field plants, it is biologically reasonable to assume that multiple infection sites could be distributed all over the host. Targeted plants could be exposed multiple times during vector movement or feeding, as vector-borne pathogens have been found capable of even altering the
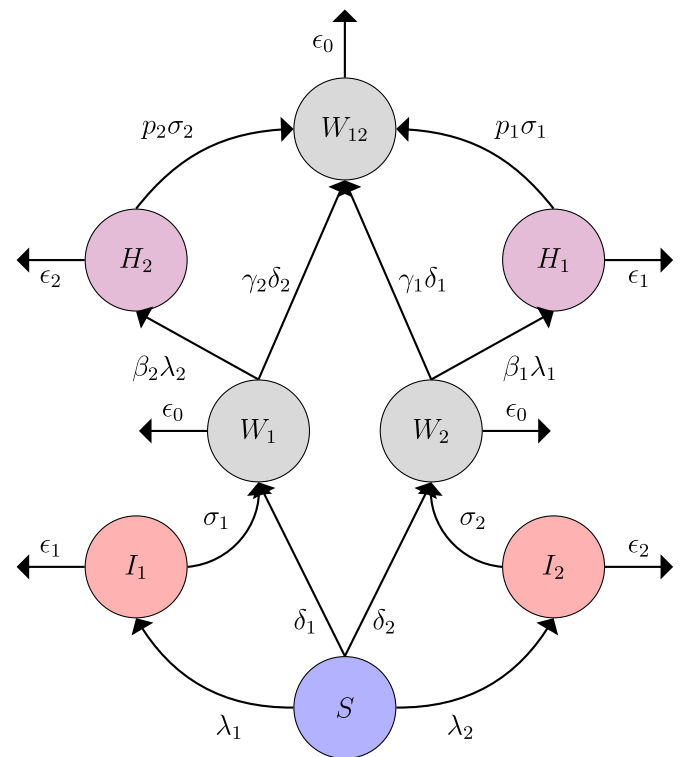

Fig. 1. A diagram of interactions between two competing viruses and the corresponding plant immune response. Here S denotes the susceptible cells, $I_{1,2}$ and $W_{1,2}$ are the infected and the warned cells for each virus, respectively. Warned cells subsequently infected by a primary or secondary virus are denoted by H_1 and H_2. Finally, W_{12} denotes the super-protected cells immune to both viruses. The arrows indicate the rates of transitions from one category of cells to another.

phenotypes of their hosts and vectors in such a way that the frequency and the nature of interactions between them promotes the transmission of the disease (Mauck et al., 2010; Moreno-Dela-fuente et al., 2013). Furthermore, all plant cells are connected through plasmodesmata, the phloem and the xylem vessels responsible for resource translocation (Lalonde et al., 2004), and these pathways can also be used by viruses for systemic infections of their host (Opalka et al., 1998; Wan et al., 2015).

Plant growth models can generally be divided into two classes: the ones where cell populations are allowed to exhibit unbounded growth, and the ones that assume a certain asymptotic final size due to finite resources or ontogenetic changes, like flowering of the plant. Asymptotic growth models are more favourable in the studies which consider the entire lifespan of the plant (Paine et al., 2012; Heinen, 1999). Hence, we will describe plant growth by the logistic growth function with a linear growth factor r and a carrying capacity K, with all cell populations contributing to the competition term, as has been effectively done in other models of immune response to infections, such as influenza (Tridane and Kuang, 2010), HIV (Perelson and Nelson, 1999) and HBV (Ciupe et al., 2007).

Once a plant becomes infected, infected cell populations $I_1(t)$ and $I_2(t)$ produce new infections by infecting susceptible (healthy) cells at rates λ_1 and λ_2, respectively. Due to various metabolic changes and loss of functions that occur after a viral takeover, the lifespan of infected cells is normally shorter than that of healthy cells, as characterised by higher death rates ϵ_1 and ϵ_2. Another possible explanation of a premature death of infected cells is given by the hypersensitive response of the plant, where infected cells would be programmed to a premature death in order to avoid the spread of the infection and to isolate the infectious site (Zvereva and Pooggin, 2012; Hinrichs et al., 1998; Fritig and Kauffmann,

2007).

In this paper we will assume that a viral infection does not always have a devastating effect on the cell, and hence it is possible for infected cells to recover before experiencing critical damage. Such recovered cells, denoted by $W_1(t)$ and $W_2(t)$, will be considered immune to the corresponding viruses in a sense that they are no longer infectious. The recovery rates σ_1 and σ_2 represent cumulative effects of the two events mentioned above and represent the rates of transition from infected to warned compartments for each of the two viruses. As described in the Introduction, one of the core mechanisms of the plant immune system is the ability to spread a warning signal that is initiated from infectious sites to other parts of the plant and to protect neighbouring cells against the imminent virus infection. For the sake of simplicity, the cells that have acquired immunity via this warning signal are also included in $W_1(t)$ and $W_2(t)$ populations. We assume that infected cells initiate and spread the warning signal to healthy cells at the rate δ_1 and δ_2, respectively. Cells that have been the recipients of the propagating signal for both viruses or have recovered from both a primary and a subsequent secondary infection will be represented by the super-protected population of cells $W_{12}(t)$ taken to be immune to both viruses. Thus, warned cells $W_1(t)$ and $W_2(t)$ will be recruited to the super-protected population $W_{12}(t)$ at modified warning rates $\gamma_2\delta_2$ and $\gamma_1\delta_1$, respectively. It is important to note that the resistance to the disease is almost always accompanied by a reduction of fitness normally represented by a reduced reproduction capability of cells (Burdon and Thrall, 2003; Tian et al., 2003). In this model we assume no fitness cost in the traditional way, however, immune cells might also experience a shorter lifespan compared to susceptible cells, and, therefore, some fitness cost can be implemented by choosing the appropriate death rate ϵ_0 for super-protected cells $W_{12}(t)$.

The warned cells that have acquired immunity to a primary infection but have successfully been infected by a secondary infection will be denoted by $H_i(t)$, where the index $i=1,2$ signifies the current infectious state of the cell. Because of their acquired immunity to one of the viruses, these cells may be less or more resistant to the other virus. If the degree of homology between the two viruses is high, i.e the two viruses are closely immunologically related, it would imply that a cell which is immune or highly resistant to one of the viruses would express the same amount of resistance to both of viruses. On the other hand, if the two viruses are not related, it is reasonable to assume that expressing an antiviral resistance to one of the viruses could induce a susceptibility to a secondary non-related infection by reducing the efficacy of the immune response.

From a biological perspective there could be a limited number of components in the cell that can be used to mount an immune response against a viral infection. For example, unless a cell is warned by both propagating signals, it might be the case that all components able to form antiviral complexes within the cell are being used to prepare only for a single infection, or that there might not be enough components in general to mount a sufficient immune response to both infections simultaneously. Moreover, chemical changes within the cell introduced during the primary infection and the corresponding immune response could potentially provide more favourable conditions in which the secondary infection is established more easily. In light of these observations, the infectious cells $H_1(t)$ and $H_2(t)$ will infect other cells at the modified infection rates $a_1\lambda_1$ and $a_2\lambda_2$ to account for either enhanced ($a_{1,2} > 1$) or reduced ($a_{1,2} < 1$) viral transmissibility. Similarly, we introduce the susceptibility modifiers β_1 and β_2 for the warned cells $W_2(t)$ and $W_1(t)$, respectively, which will be assumed to be either susceptible ($\beta_{1,2} > 1$) or resistant ($\beta_{1,2} < 1$) to the virus

agains which they have not yet acquired immunity. To account for a prior infection, the recovery rates of cells H_i are modified by the factors p_{ie}, so these cells are recruited into the super-protected population at rates $p_1\sigma_1$ and $p_2\sigma_2$, respectively. Therefore, in this model the parameters that define viral cooperation will be the modifiers i.e a_i, β_i and p_{ie} which can be interpreted as either functions of the antigenic distance or other specific relation between two viruses. For simplicity, we will ignore the possibility of random mutations, so that these modifiers will remain constant.

Under the above assumptions, the model describing the dynamics of plant immune response to two viral infections can be written as follows,

$$\frac{dS}{dt} = r\hat{S}\left(1 - \frac{N}{K}\right) - S\left[(\lambda_1 + \delta_1)I_1 + (\lambda_2 + \delta_2)I_2 + a_2\lambda_2H_2 + a_1\lambda_1H_1\right],$$

$$\frac{dI_1}{dt} = I_1(\lambda_1 S - \sigma_1 - \epsilon_1) + a_1\lambda_1H_1S,$$

$$\frac{dI_2}{dt} = I_2(\lambda_2 S - \sigma_2 - \epsilon_2) + a_2\lambda_2H_2S,$$

$$\frac{dW_1}{dt} = I_1(\sigma_1 + \delta_1 S) - W_1\left[\epsilon_0 + (\beta_2\lambda_2 + \gamma_2\delta_2)I_2 + \beta_2a_2\lambda_2H_2\right],$$

$$\frac{dW_2}{dt} = I_2(\sigma_2 + \delta_2 S) - W_2\left[\epsilon_0 + (\beta_1\lambda_1 + \gamma_1\delta_1)I_1 + \beta_1a_1\lambda_1H_1\right],$$

$$\frac{dH_1}{dt} = W_2(\beta_1\lambda_1I_1 + \beta_1a_1\lambda_1H_1) - H_1(\epsilon_1 + p_1\sigma_1),$$

$$\frac{dH_2}{dt} = W_1(\beta_2\lambda_2I_2 + \beta_2a_2\lambda_2H_2) - H_2(\epsilon_2 + p_2\sigma_2),$$

$$\frac{dW_{12}}{dt} = p_1\sigma_1H_1 + p_2\sigma_2H_2 + \gamma_2\delta_2I_2W_1 + \gamma_1\delta_1I_1W_2 - \epsilon_0W_{12}, \quad (1)$$

where $\hat{S}(t) = S(t) + W_1(t) + W_2(t) + W_{12}(t)$, and $N(t) = S(t) + I_1(t) + I_2(t) + W_1(t) + W_2(t) + H_1(t) + H_2(t) + W_{12}(t)$ is the total population of plant cells. As a first step of the analysis, we establish well-posedness of the system (1).

Theorem 2.1. *The model (1) with initial conditions*

$$S(0) > 0, \quad I_1(0) \geq 0, \quad I_2(0) \geq 0, \quad W_1(0) \geq 0, \quad W_2(0) \geq 0,$$
$$H_1(0) \geq 0, \quad H_2(0) \geq 0, \quad W_{12}(0) \geq 0,$$

and $N(0) = N_0 < K$ is well-posed, i.e. its solutions remain non-negative and bounded for all $t \geq 0$.

Proof. Let T_2 be a period of time, such that $N(t) < K$ for $t \in [0, T_2]$, and suppose $T_1 \leq T_2$ is the first time such that $S(T_1) = 0$. This implies that

$$\dot{S}(T_1) = r(W_1 + W_2 + W_{12})[1 - (W_1 + W_2 + W_{12} + I_1 + I_2)/K] \geq 0,$$

hence, for any $0 \leq t \leq T_2$, we have that $S(t) \geq 0$. For the remaining variables, considering any positive time t, if for any $i=1,2$ we have that $I_i(t) = 0$, this implies that $\dot{I}_i(t) = a_i\lambda_iH_i \geq 0$, thus $I_i(t)$ must be non-negative for all times. Likewise, for both $W_i(t) = 0$ we obtain $\dot{W}_i(t) = I_i(\sigma_i + \delta_iS) \geq 0$ which shows that $W_i(t) \geq 0$. If $H_i(t) = 0$, we have $\dot{H}_i(t) = W_j\beta_i\lambda_iI_i \geq 0$ with $1 \leq i \neq j \leq 2$. Finally, for $W_{12}(t) = 0$, we have that $\dot{W}_{12}(t) \geq 0$. Thus, all variables remain non-negative for $t \in [0, T_2]$.

We now prove, by contradiction, that, in fact, $N(t) < K$ for all $t \geq 0$. Assume, for a contradiction, that there is a first time $T_2 > 0$ at which the inequality $N(t) < K$ ceases to hold. Since T_2 is the first such time, $N(T_2) = K$ and $\dot{N}(T_2) \geq 0$. As has been shown earlier, all state variables are non-negative at $t = T_2$. Adding up all equations of the system (1) yields

$$\frac{dN}{dt} = r\hat{S}(1 - N/K) - \epsilon_1I_1 - \epsilon_2I_2 - \epsilon_0(W_1 + W_2) - \epsilon_1H_1 - \epsilon_2H_2$$
$$- \epsilon_0W_{12}, \quad (2)$$

Since at $t = T_2$ we have that $N(T_2) = K$, the last equation gives $\dot{N}(T_2) < 0$, which is a contradiction, unless $I_1(T_2) = I_2(T_2) = W_1(T_2) = W_2(T_2) = H_1(T_2) = H_2(T_2) = W_{12}(T_2) = 0$. But in this exceptional case, the initial value theorem for ODEs, applied to the last 7 equations of system (1) with S considered as a pre-scribed function, yields that $I_1(t) = I_2(t) = W_1(t) = W_2(t) = H_1(t) = H_2(t) = W_{12}(t) = 0$ for all $t > T_2$ and the equation for $S(t)$ (the first equation of the system) reduces to the logistic equation $\dot{S} = rS(1 - S/K)$. Thus, for any $t \geq T_2$, we have $0 < S(t) \leq K$, which completes the proof. $\square$

To simplify the model and reduce the number of free para-meters, we non-dimensionalise the system (1) by introducing new dimensionless variables

$$\tau = rt, \quad u_1 = \frac{S}{K}, \quad u_2 = \frac{I_1}{K}, \quad u_3 = \frac{I_2}{K}, \quad u_4 = \frac{W_1}{K},$$

$$u_5 = \frac{W_2}{K}, \quad u_6 = \frac{H_1}{K}, \quad u_7 = \frac{H_2}{K}, \quad u_8 = \frac{W_{12}}{K},$$

and for $i = 1, 2$, parameters

$$L_i = \frac{\lambda_i}{r}, \quad d_i = \frac{K\delta_i}{r}, \quad e_i = \frac{\epsilon_i}{r}, \quad s_i = \frac{\sigma_i}{r}, \quad e_0 = \frac{\epsilon_0}{r}.$$

This gives the following modified system

$$\frac{du_1}{d\tau} = \hat{u}_1(1 - \hat{N}) - u_1\left[(L_1 + d_1)u_2 + (L_2 + d_2)u_3 + a_1L_1u_6 + a_2L_2u_7\right],$$

$$\frac{du_2}{d\tau} = L_1\left(a_1u_6 + u_2\right)u_1 - u_2\left(e_1 + s_1\right),$$

$$\frac{du_3}{d\tau} = L_2\left(a_2u_7 + u_3\right)u_1 - u_3\left(e_2 + s_2\right),$$

$$\frac{du_4}{d\tau} = u_2\left(d_1u_1 + s_1\right) - u_4\left[(\beta_2L_2 + \gamma_2d_2)u_3 + \beta_2a_2L_2u_7 + e_0\right],$$

$$\frac{du_5}{d\tau} = u_3\left(d_2u_1 + s_2\right) - u_5\left[(\beta_1L_1 + \gamma_1d_1)u_2 + \beta_1a_1L_1u_6 + e_0\right],$$

$$\frac{du_6}{d\tau} = \beta_1L_1\left(a_1u_6 + u_2\right)u_5 - u_6\left(p_1s_1 + e_1\right),$$

$$\frac{du_7}{d\tau} = \beta_2L_2\left(a_2u_7 + u_3\right)u_4 - u_7\left(p_2s_2 + e_2\right),$$

$$\frac{du_8}{d\tau} = \gamma_1d_1u_2u_5 + \gamma_2d_2u_3u_4 + p_1s_1u_6 + p_2s_2u_7 - e_0u_8, \tag{3}$$

where $\hat{u}_1 = u_1 + u_4 + u_5 + u_8$ and $\hat{N} = \hat{u}_1 + u_2 + u_3 + u_6 + u_7$.

3. Steady states

It is straightforward to see that independently of the values of parameters, the system (3) always admits a trivial steady state

$$E_0 = (0, 0, 0, 0, 0, 0, 0, 0), \tag{4}$$

and a disease-free steady state given by

$$E_{DF} = (1, 0, 0, 0, 0, 0, 0, 0). \tag{5}$$

Looking for steady states of the system (3) $u_2 = 0$ and $u_{1,3} \neq 0$, gives $u_4 = u_6 = u_7 = u_8 = 0$. Substituting these values in other equations of system (3) gives a one-virus endemic steady state

$$E_2 = (u_1^*, 0, u_3^*, 0, u_5^*, 0, 0), \tag{6}$$

where

$$u_1^* = \frac{e_2 + s_2}{L_2}, \quad u_3^* = \frac{-c_1(u_1^*) - \sqrt{c_1^2(u_1^*) - 4c_2(u_1^*)c_0(u_1^*)}}{2c_2(u_1^*)},$$

$$u_5^* = A(u_1^*)u_3^*,$$

with

$$A(u_1^*) = \frac{d_2u_1^* + s_2}{e_0}, \quad B = L_2 + d_2, \quad c_0(u_1^*) = u_1^*(1 - u_1^*),$$

$$c_1(u_1^*) = A(u_1^*) - u_1^*[2A(u_1^*) + B + 1], \quad c_2(u_1^*) = -A(u_1^*)[A(u_1^*) + 1].$$

The steady state E_2 is biologically feasible, as long as the condition $e_2 + s_2 < L_2$ holds.

Proceeding in a similar manner, one can find a one-virus en-demic steady state E_1 corresponding to the presence of virus 1 only. This steady state is explicitly given by

$$E_1 = (\tilde{u}_1^*, u_2^*, 0, u_4^*, 0, 0, 0), \tag{7}$$

where now

$$\tilde{u}_1^* = \frac{e_1 + s_1}{L_1}, \quad u_2^* = \frac{-\tilde{c}_1(\tilde{u}_1^*) - \sqrt{\tilde{c}_1^2(\tilde{u}_1^*) - 4\tilde{c}_2(\tilde{u}_1^*)\tilde{c}_0(u_1^*)}}{2\tilde{c}_2(u_1^*)},$$

$$u_4^* = \tilde{A}(\tilde{u}_1^*)u_2^*,$$

with

$$\tilde{A}(\tilde{u}_1^*) = \frac{d_1\tilde{u}_1^* + s_1}{e_0}, \quad \tilde{B} = L_1 + d_1, \quad \tilde{c}_0(\tilde{u}_1^*) = \tilde{u}_1^*(1 - \tilde{u}_1^*),$$

$$\tilde{c}_1(\tilde{u}_1^*) = \tilde{A}(\tilde{u}_1^*) - \tilde{u}_1^*[2\tilde{A}(\tilde{u}_1^*) + \tilde{B} + 1], \quad \tilde{c}_2(\tilde{u}_1^*)$$
$$= -\tilde{A}(\tilde{u}_1^*)[\tilde{A}(\tilde{u}_1^*) + 1].$$

This steady state is biologically feasible whenever the condition $e_1 + s_1 < L_1$ is satisfied.

Besides the disease-free and the two one-virus endemic steady states, system (3) can support one or more *syndemic* steady states characterised by the simultaneous presence of both viruses,

$$S = (u_1^*, u_2^*, u_3^*, u_4^*, u_5^*, u_6^*, u_7^*, u_8^*). \tag{8}$$

To find this steady state, let us introduce auxiliary variables and functions

$$u_0 = \min_{i=1,2}\left(\frac{e_i + s_i}{L_i}\right), \quad F_i(x) = -\frac{(L_ix - e_i - s_i)}{L_ia_ix}, \quad i = 1, 2,$$

$$\Delta_i(x) = \beta_iL_i(F_i(x)a_i + 1) + d_i\gamma_i, \quad G_i(x) = d_ix + s_i, \quad i = 1, 2, \tag{9}$$

which allow us to express all steady state variables through u_1^* in the following way:

$$u_4^* = \frac{F_2(u_1^*)(p_2s_2 + e_2)}{\beta_2L_2[a_2F_2(u_1^*) + 1]}, \quad u_5^* = \frac{F_1(u_1^*)(p_1s_1 + e_1)}{\beta_1L_1[a_1F_1(u_1^*) + 1]},$$

$$u_2^* = \frac{e_0u_4^*[\Delta_2(u_1^*)u_5^* + G_2(u_1^*)]}{G_1(u_1^*)G_2(u_1^*) - \Delta_1(u_1^*)\Delta_2(u_1^*)u_4^*u_5^*},$$

$$u_3^* = \frac{e_0u_5^*[\Delta_1(u_1^*)u_4^* + G_1(u_1^*)]}{G_1(u_1^*)G_2(u_1^*) - \Delta_1(u_1^*)\Delta_2(u_1^*)u_4^*u_5^*},$$

$$u_6^* = u_2^*F_1(u_1^*), \quad u_7^* = u_3^*F_2(u_1^*),$$

$$u_8^* = \frac{d_1\gamma_1u_2^*u_5^* + d_2\gamma_2u_3^*u_4^* + p_1s_1u_6^* + p_2s_2u_7^*}{e_0}.$$

Substituting these expressions into the equation

$$\hat{u}_1^*(1 - \hat{N}) - u_1^*\left[(L_1 + d_1)u_2^* + (L_2 + d_2)u_3^* + a_1L_1u_6^* + a_2L_2u_7^*\right] = 0,$$

yields a polynomial equation for u_1^*, whose roots gives possible candidates for the syndemic steady state. This state is biologically feasible if

$$0 < u_1^* < u_0, \quad G_1(u_1^*)G_2(u_1^*) - \Delta_1(u_1^*)\Delta_2(u_1^*)u_4^*u_5^* > 0.$$

Linearising system (3) near the trivial steady state E_0 gives the following characteristic equation for eigenvalues μ:

$$(\mu - 1)(\mu + e_0)^3 \prod_{i=1}^{2} (\mu + e_i + s_i)(\mu + p_i s_i + e_i) = 0.$$

Since one of the roots is $\mu = 1$, this implies that the trivial steady state is always unstable, and, therefore, it is impossible for all cell populations to die out. Linearisation near the disease-free steady state E_{DF} has a characteristic equation

$$(\mu + 1)(\mu + e_0)^3 \prod_{i=1}^{2} (p_i s_i + \mu + e_i)(\mu - L_i + e_i + s_i) = 0, \qquad (10)$$

implying that the disease-free steady state E_{DF} is linearly asymptotically stable, provided $u_0 > 1$, with u_0 defined in (9). In epidemiology, one of the most common and efficient techniques for establishing criteria for onset of epidemic outbreaks is analysis of the *basic reproduction number* R_0, defined as the average number of secondary infections produced by a single infected individual in a totally susceptible population (Hethcote, 2000; Dietz, 1993; van den Driessche and Watmough, 2008; Heesterbeek and Dietz, 1996). This quantity can be derived in a number of ways, e.g. using the next generation approach (van den Driessche and Watmough, 2008), we define the basic reproduction number for each of the viruses as follows

$$R_{01} = \frac{L_1}{e_1 + s_1} \quad R_{02} = \frac{L_2}{e_2 + s_2}, \qquad (11)$$

and denote $R_0 = \max\{R_{01}, R_{02}\} = u_0^{-1}$. Then, the disease-free steady state E_{DF} is linearly asymptotically stable if $R_0 < 1$. This result means that a complete recovery from both viral infections depends on the efficacy of RNA interference from local induction, i. e the ability of the host cell to target and degrade viral RNA in order to inhibit viral multiplication, and also on whether infected cells reach their limited lifespan faster than they can spread the disease for each virus, respectively. Furthermore, since the basic reproduction number R_0 does not depend on the transmissibility ($a_{1,2}$) or susceptibility ($\beta_{1,2}$) modifiers, this implies that the interactions between the two viruses during the host co-infection cannot cause both viruses to become extinct. On the other hand, the modifiers may determine whether both viruses, or only one of them will survive.

Characteristic equation of linearisation near the endemic steady state E_2 can be factorised into

$$X_1(\mu)X_2(\mu)X_3(\mu) = 0, \qquad (12)$$

where

$$X_1(\mu) = (\mu + e_0)(p_2 s_2 + \mu + e_2)\left[u_3^*(L_2\beta_2 + d_2\gamma_2) + \mu + e_0\right],$$

$$X_2(\mu) = \mu^2 + x_{21}\mu + x_{20}, \quad X_3(\mu) = \mu^3 + x_{32}\mu^2 + x_{31}\mu + x_{30},$$

and

$$x_{21} = s_1(p_1 + 1) + 2e_1 - L_1(a_1\beta_1 u_5^* + u_1^*),$$

$$x_{20} = (p_1 s_1 + e_1)(e_1 + s_1 - L_1 u_1^*) - L_1 a_1 \beta_1(e_1 + s_1)u_5^*,$$

$$x_{32} = 2u_1^* + (L_2 + d_2 + 1)u_3^* + 2u_5^* + e_0 - 1,$$

$$x_{31} = d_1(u_3^*)^2 + \left[(L_2 + d_2)[u_1^*(L_2 + 1) + u_5 + e_0] + d_2(u_1^* + u_5^* - 1) + e_0\right]u_3^*$$
$$\quad + e_0(2u_1^* + 2u_5^* - 1),$$

$$x_{30} = L_2 u_3^*\left[d_2 u_1^*(2(u_1^* + u_5^*) + u_3^* + e_0 - 1) + u_1^* e_0(L_2 + 1) + s_2(2u_1^* + u_3^* - 1)\right]$$
$$\quad + L_2 u_3^* + u_5^*(e_0 + 2s_2). \qquad (13)$$

Since all system parameters are strictly positive, the roots of $X_1(\mu)$ are all real and negative. By the Routh-Hurwitz criterion we have that all roots of $X_2(\mu)$ lie in the left complex half-plane if the coefficients x_{21} and x_{20} are positive, which translates into the requirements

$$u_5^* < \frac{(s_1 p_1 + e_1) + (s_1 + e_1 - L_1 u_1^*)}{L_1 a_1 \beta_1} := u_A, \quad \text{and}$$

$$u_5^* < \frac{(s_1 p_1 + e_1)(s_1 + e_1 - L_1 u_1^*)}{L_1 a_1 \beta_1 (e_1 + s_1)} := u_B. \qquad (14)$$

Since u_5^* must be positive, we require that $u_1^* < (s_1 + e_1)/L_1$. Additionally, since $s_1 + e_1 - L_1 u_1^* < s_1 + e_1$, we have

$$u_A = \frac{s_1 p_1 + e_1}{L_1 a_1 \beta_1} + \frac{s_1 + e_1 - L_1 u_1^*}{L_1 a_1 \beta_1} > \frac{s_1 p_1 + e_1}{L_1 a_1 \beta_1},$$

$$u_B = \frac{s_1 p_1 + e_1}{L_1 a_1 \beta_1}\frac{s_1 + e_1 - L_1 u_1^*}{e_1 + s_1} < \frac{s_1 p_1 + e_1}{L_1 a_1 \beta_1}, \qquad (15)$$

implying $u_B < u_A$. Hence, the roots of $X_2(\mu)$ have a negative real part, provided

$$u_1^* < \frac{s_1 + e_1}{L_1} = \tilde{u}_1^* \quad \text{and} \quad u_5^* < u_B.$$

This also implies that a necessary condition for the stability of the endemic steady state E_2 is the intuitively natural result that the two basic reproduction numbers defined in (11) must satisfy $R_{02} > R_{01}$.

Applying the Routh-Hurwitz criterion to the cubic polynomial $X_3(\mu)$ gives that all roots of this polynomial have negative real parts, as long as x_{32}, x_{31} and x_{30} are positive and satisfy the condition $x_{32}x_{31} > x_{30}$. It is important to note that stability of the endemic steady state E_2 does not depend on the susceptibility and transmissibility modifiers a_2 and β_2. From a biological perspective, this suggests that the capability of the second virus to survive as a single infection is irrelevant from the point of view of its ability to infect cells that are chemically altered and are immune to the first virus. On the other hand, the ability of viruses d_i to trigger a warning signal appears to control whether they can exclude each other or co-exist in a stable equilibrium. Hence, we have proved the following result.

Theorem 3.1. *For the endemic steady state* $E_2 = (u_1^*, 0, u_3^*, 0, u_5^*, 0, 0)$ *with* $u_1^* = (e_2 + s_2)/L_2, u_3^*$ *and* u_5^* *given in* (6), *let* x_{30}, x_{31}, x_{32} *and* u_B *be defined by* (13) *and* (15), *respectively. Then the steady state* E_2 *is linearly asymptotically stable if and only if the following conditions hold.*

(1) $0 < u_5^* < u_B$,
(2) $x_{30} > 0, x_{31} > 0, x_{32} > 0$,
(3) $x_{32}x_{31} > x_{30}$.

Remark. The result of this **Theorem** can be applied to the analysis of stability of the endemic steady state E_1 by swapping parameter indices 1 with 2, and replacing variables u_3^* and u_5^* with u_2^* and u_4^*, respectively, as a consequence of the model symmetry. Unlike some other models of multi-strain/multi-virus infections (Gupta and Anderson, 1999; Allen et al., 2003; Castillo-Chavez et al., 1996), the complexity of the model (3) prevents one from expressing the conditions for stability of single-virus or co-existence equilibria in a closed form depending only on two basic reproduction numbers.

Since the syndemic steady state S cannot be found in a closed form, it does not prove possible to derive analytical conditions for stability of this steady state. Hence, to understand how stability changes with parameters, one has to resort to numerically compute eigenvalues of the Jacobian of the linearisation of system (3) near the steady state .

4. Numerical stability analysis and simulations

Due to RNAi being a very complicated multi-component

process, obtaining accurate parameters values to be used in a mathematical model is extremely difficult and often impractical, as some parameters cannot currently be measured, or even when they do, there is a very wide variability in the reported values (Melnyk et al., 2011; Liang et al., 2012; Himber and Dunoyer, 2015). Parameter values that define viral properties and modifiers in the context of this study are equally problematic to obtain, as one would require virus-specific information about both the cell-to-cell and long-distance transmission of the virus. For example, in the case of the Tobacco mosaic virus, the infection can on average spread from one cell to another every 3–4 h depending on the strain of the virus and the temperature (Kawakami et al., 2004), and although this information can provide some intuition about parameter values, it is not sufficient for estimating the actual infection rate.

To better understand the effects of different parameters on feasibility and stability of different steady states of the system (3), we use **Theorem 3.1** and numerical computation of eigenvalues to identify parameter regions associated with existence and stability of all steady states. To this end, we start with baseline parameter values given in Table 1 and allow some of the parameters to vary. Since model (3) has quite a large number of different parameters, below we present the results for only some parameter combinations that illustrate the diversity of possible scenarios, and qualitatively similar results can be obtained when other parameters are varied. Plotting the percentages of infected cells for each steady state in the same parameter space allows us to investigate possible changes in the magnitude of the infected cell population between different steady states.

Figs. 2, 3 and 4 illustrate earlier analytical conclusions that the two endemic steady states E_1 and E_2 are only feasible and stable if the recovery/death rates of infected cells are sufficiently low. On the other hand, one expects that a virus can only survive if its infection rate is adequately high, as observed in Fig. 2(d) and Fig. 4 (a). If either one of the recovery/infection rates is below or above a certain threshold, it is easy to see that the syndemic steady state disappears, and only one of the two viruses survives. However, Fig. 2(a), (c) and 3(c), together with additional computations not shown here, suggest that by increasing parameters $a_{1,2}$, i.e the transmissibility modifiers, or the susceptibility modifiers $b_{1,2}$, the system can generally move from one of the endemic steady states to a stable syndemic equilibrium. This suggests that the most competitive viral strain, which under different circumstances would be capable of excluding a secondary infection, might instead facilitate the survival of a secondary strain. Cells that have been chemically altered by the immune response to the more aggressive strain can now serve as ideal targets in which the second strain could proliferate. Since for the fixed values of other

Table 1
Table of baseline values of parameters in system (3).

Dimensionless parameters	Biological meaning	Baseline value
$L_{1,2}$	Infection rate	1.5
$s_{1,2}$	Recovery rate	0.5
$d_{1,2}$	Propagation rate	0.05
$a_{1,2}$	Transmissibility modifier (after secondary infection)	1
$\beta_{1,2}$	Susceptibility modifier (after primary infection)	1
$\gamma_{1,2}$	Acquired secondary immunity modifier	0.5
e_0	Natural death rate	0.3
$e_{1,2}$	Infected cell death rate	0.6
$p_{1,2}$	Recovery modifier	0.2

parameters, infection rates L_1 and L_2 are proportional to the two basic reproduction numbers, R_{01} and R_{02}, respectively, Fig. 4 (a) effectively is equivalent to figures demonstrating the dependence of steady states on basic reproduction numbers in two-strain models of infectious diseases (Gupta et al., 1996; Andreasen et al., 1997).

Fig. 2(d) and 3(a) show that when one of the recovery modifiers $p_{1,2}$ is increased, the system can move from the syndemic to one of the endemic equilibria $E_{1,2}$, thus behaving in a qualitatively opposite way to an increase of the corresponding parameter pair $\{a_i, \beta_i\}$. This occurs when cells with acquired immunity to one of the viruses are subsequently infected with another virus but have a faster recovery. As this reduces the overall spread of the secondary infection, it will inevitably allow the primary virus to dominate and eventually be the sole survivor in the host. In Fig. 2 (b) one observes that by increasing the dimensionless warning rate d_2 we can move from a parameter region where only the endemic steady state E_2 is feasible and stable (a grey region) to a region, where the syndemic equilibrium is also stable (an orange region). This suggests that the plant immune response to the second virus can establish conditions that are more favourable to the first virus. Thus, in the case of a double infection, it is possible for a viral infection to persevere in the presence of the host's immune response despite being unable to do so as a single infection. This means that the propagating component of the immune response plays a significant role in the interactions between two viruses and can dictate whether both of them can survive in a single host.

Recall that in model (3), the two viruses are considered to cooperate with each other when $a_i, \beta_i > 1$, have a neutral relationship when $a_i, \beta_i = 1$, and "antagonise" each other when $a_i, \beta_i < 1$, $i = 1,2$. One should also note the existence of other more complicated scenarios as each of a_1, a_2, β_1 and β_2 can be less than, greater than or equal to one. For example, if $a_1, \beta_1 > 1$ and $0 < a_2, \beta_2 < 1$ then, cooperation of the two viruses will be considered to benefit mostly the first virus, thus being unequal. On the other hand, for $a_2, \beta_2 > 1$ and $a_1, \beta_1 = 0$, the relationship is completely one-sided in favour of the second virus. Fig. 5(a) and (b) suggest that the biological interactions between different viruses may sometimes disproportionately favour one of the viruses and decrease the potency of the second infection, that is to say that one of the viruses experiences less spread during a co-infection when compared to its single-virus infected steady state. This is clearly evident in Fig. 5(b): for $\beta_2 \leq 0.87$ only the first virus is present, whereas for $\beta_2 > 0.87$ the system moves into the syndemic steady state where now both viruses are able to survive, but the first virus is not as widely spread as before. One should note that this result comes at the cost of increasing the total number of infected cells, suggesting that it might not always be the preferable outcome for the plant. Similarly, Fig. 5(a) shows that for small values of the transmissibility modifier a_1 combined with a higher infection rate $L_2 > L_1$ (which also implies $R_{02} > R_{01}$), only the second virus is able to survive in the host. As the value of a_1 increases, the picture changes, and the system moves to a syndemic steady state, where not only both of the viruses are able to survive, but given sufficiently high value of a_1, the first virus can become dominant. This also suggests that increasing a_i is qualitatively interchangeable increasing β_j for $j \neq i$. Fig. 5(c) and (d) show how depending on the level of cooperation between the two viruses, i.e for sufficiently high values of a_1 and a_2, it can be beneficial for the viruses to coexist, as they can both infect a bigger biomass of the host compared to their respective one-virus steady states, possibly resulting in a chronic condition that is more severe. These results show that sufficient levels of mutual cooperation between two viruses promote their virulence and ensure that neither of them becomes eradicated, which eventually leads to a persistent double infection
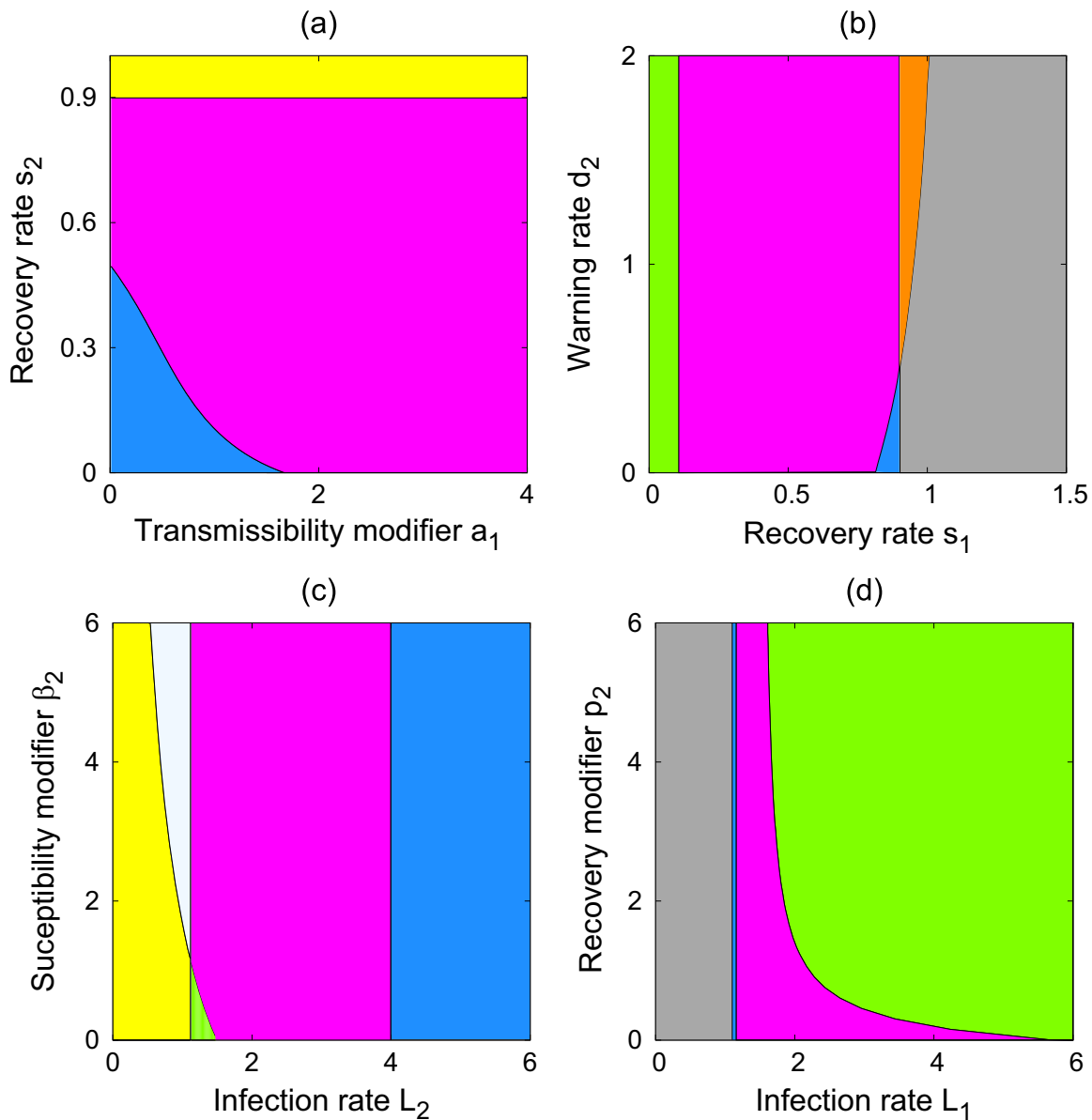
Fig. 2. Stability of steady states of the system (3) with parameters from Table 1. Green and blue indicate regions where both endemic steady states E_1 and E_2 are feasible, but only E_1 or E_2 is stable, respectively. Magenta shows the region where all three infected steady states are feasible, but only the syndemic steady state S is stable. Yellow is the area where only E_1 is feasible and stable, whereas grey is the area where only E_2 is feasible and stable. White and orange is where the syndemic steady state is stable, whereas E_1 or E_2, respectively, is also feasible (and unstable). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with parameter values determining the magnitude of each infection.

If the cooperation between the two viruses is unequal or one-sided, it is possible that the least benefited virus will experience less spread compared to its single-virus infected steady state. To investigate scenarios where both viruses "antagonise" each other we solved the system at a_i, $\beta_i = 0.5$, $i=1,2$. One result is given in Fig. 6(a), and it shows that increasing β_2 decreases the presence of the first virus, but similarly to our previous results it increases the overall level of infection. The most interesting case is shown in Fig. 6(b), where adequately increasing the warning rate d_2, not only the percentage of cells infected with the second virus goes down, but also the total number of infected cells is reduced. One also observes in this Figure that although the number of cells infected with the first virus is slowly increasing, it is still at a much lower level than what it was in the absence of the second virus, i.e

compared to the steady state E_1 which is now unstable. This situation represents an ideal scenario, where inoculating the target plant with a less harmful virus or viral strain can offer partial protection against another specific virus or strain, thus potentially minimising damage to the host.

To demonstrate different kinds of dynamics that can be exhibited by the model, we have solved the system (3) numerically for different combinations of parameters, and the results are shown in Fig. 7. Fig. 7(a) shows the solution of the model that approaches the stable syndemic steady state, with all compartments having positive values. As we discussed earlier, from a biological perspective this represents the cases where interactions between the two viruses facilitate the survival of both viral species within the same host. Fig. 7(b) and (c) illustrate situations where one of the viruses survives, while the other one is eradicated by the plant immune system, and Fig. 7(d) demonstrates the case
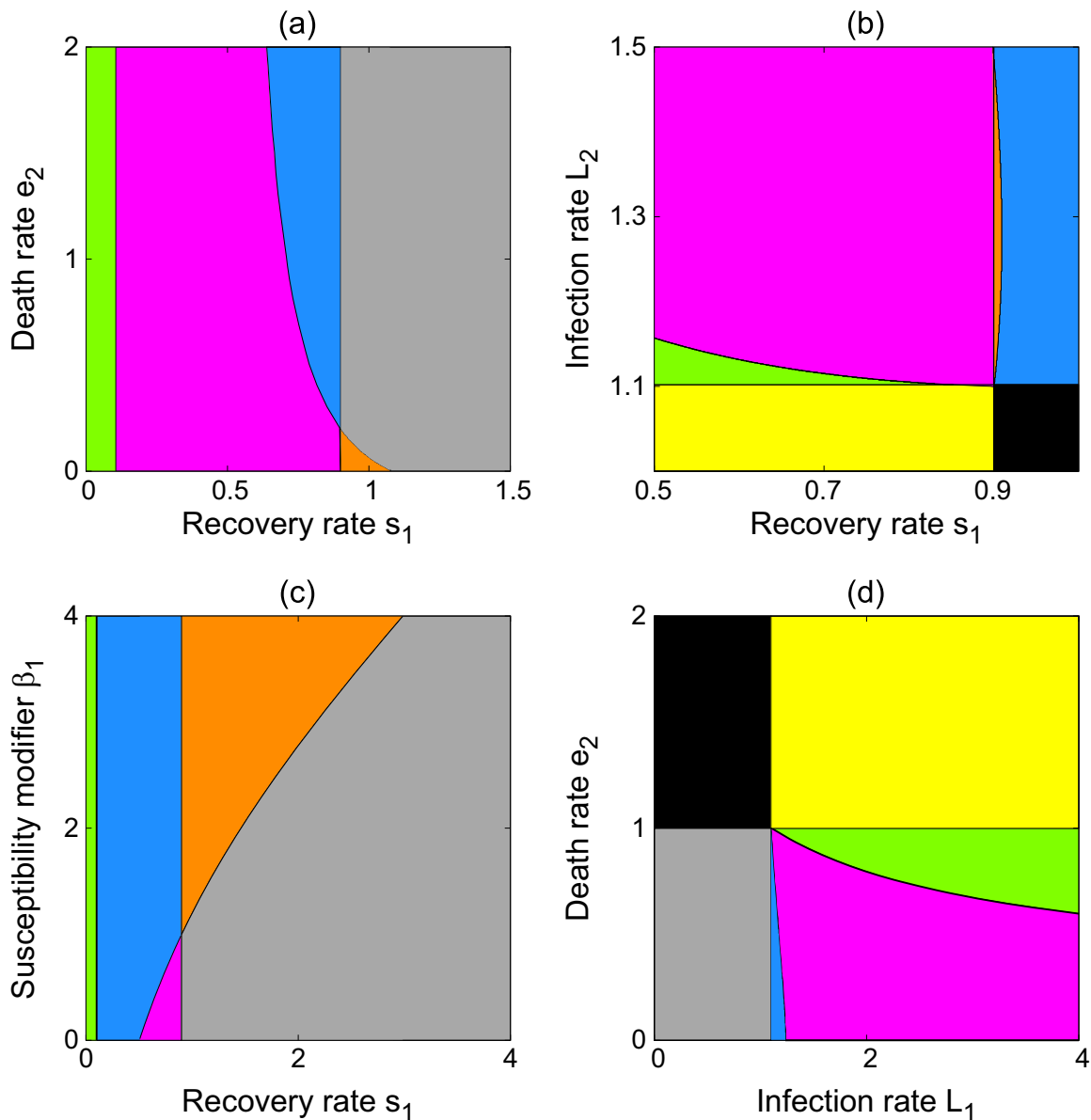
Fig. 3. Stability of the steady states of the system (3) with parameters from Table 1. Green and blue indicate regions where both endemic steady states E_1 and E_2 are feasible, but only E_1 or E_2 is stable, respectively. Magenta shows the region where all three infected steady states are feasible, but only the syndemic steady state S is stable. Yellow is the area where only E_1 is feasible and stable, whereas grey is the area where only E_2 is feasible and stable. Orange is where the syndemic steady state is stable, and E_2 is feasible but unstable. Black is the region where only the disease-free steady state is feasible and stable. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

where the plant makes a full recovery.

5. Discussion

In this paper we have derived and analysed a mathematical model of biological interactions between two viruses and a single plant host, with particular account for RNA interference.

Our results have shows that RNA interference can provide a mechanism for cross-protection, and a co-infection can either increase or decrease the overall potency of individual infections, illustrating how cross-protection or cross-enhancement can occur between the two viruses. The framework developed in this paper can be directly applicable to analysis of RNAi-mediated interactions for many combinations of plant viruses, with examples including co-infections with Soybean mosaic virus and Alfalfa mosaic virus (Malapi-Nelson et al., 2009), as well as Abutilon mosaic

virus and Cucumber mosaic virus (Wege and Siegmund, 2007). The model can also be used to obtain insights into how one could control viral diseases through cross-protection and, by extension, through gene and antiviral therapy, where genetically modified viruses are introduced to the host. Unlike the wild type strains, these modified viruses can be engineered to deliver specific therapeutic siRNA, which through the process of RNA interference would trigger immune response, thus acting as a powerful vaccination strategy (Silva et al., 2002; Caplen, 2004; Soifer et al., 2007).

To achieve greater biological realism, we have assumed that the new plant growth depends on the availability of healthy cells which can be impeded once the plant becomes infected. Stability analysis of the steady states has demonstrated the significance of different parameters of the model and showed how they dictate the dynamical behaviour exhibited by the system.

One should note that in the current model it is impossible for all cell populations to die, as there will always be some new
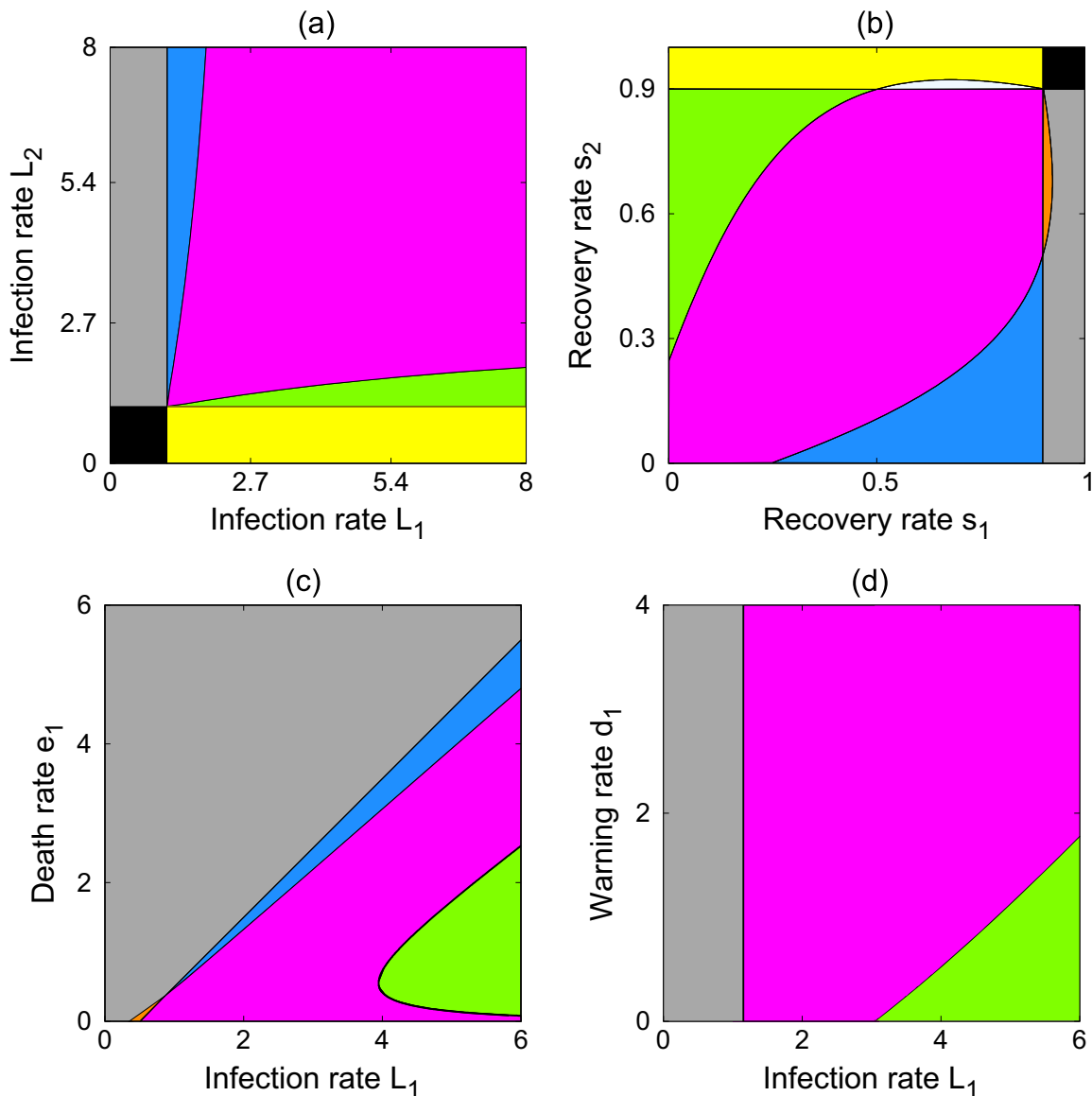
Fig. 4. Stability of steady states of the system (3) with parameters from Table 1. Green and blue indicate regions where both endemic steady states E_1 and E_2 are feasible, but only E_1 or E_2 is stable, respectively. Magenta shows the region where all three infected steady states are feasible, but only the syndemic steady state S is stable. Yellow is the area where only E_1 is feasible and stable, whereas grey is the area where only E_2 is feasible and stable. White and orange is where the syndemic steady state is stable, whereas E_1 or E_2, respectively, is also feasible (and unstable). Black is the region where only the disease-free steady state is feasible and stable. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

growth taking place to replace the parts of the plant that are lost either naturally or due to infections. This is true despite the growth penalty introduced by allocating some of the resources to infected parts of the plant. Even if a plant were to experience a severe case of stunting, it would be highly unlikely that every healthy cell would become infected, and, therefore, lead to the death of the plant. Although our model cannot capture this scenario, realistically, such events do occur quite rarely in nature.

Stability of the disease-free equilibrium and the feasibility of the two single-virus endemic steady states depends on the two basic reproduction numbers R_{01} and R_{02}. In our model these quantities are represented as functions of only the infection, recovery and death rates of infected cells for each strain, but they are not affected by the propagating component of the immune response. This suggests that a faster mobile signal can at most help the plant to recover faster (as determined by the above-mentioned factors) but, by itself it is not sufficient for a recovery. However, this picture changes when the stability of the syndemic steady

state is considered. Our results show that the warning signal plays a significant role in determining whether both viruses can persist simultaneously, and as such, it controls situations where the plant is able to support some constant level of both infections. If the two viruses are sufficiently immunologically related, so that the immune response trigged by a primary infection with a less virulent strain could induce a sufficient response against a secondary strain, then the least harmful of the two viruses becomes dominant, and the plant experiences a degree of cross-protection which may sometimes result in the increased total population of infected cells.

Analysis of the model has demonstrated that the total population of infected cells during a co-infection can sometimes, but not always, be higher than during a single infection, for which there are two possible explanations. One possibility is that the two different infections simply increase the overall rate of infection. Another aspect is that the two viruses only have to compete for susceptible cells, as there is a source of cells that might be
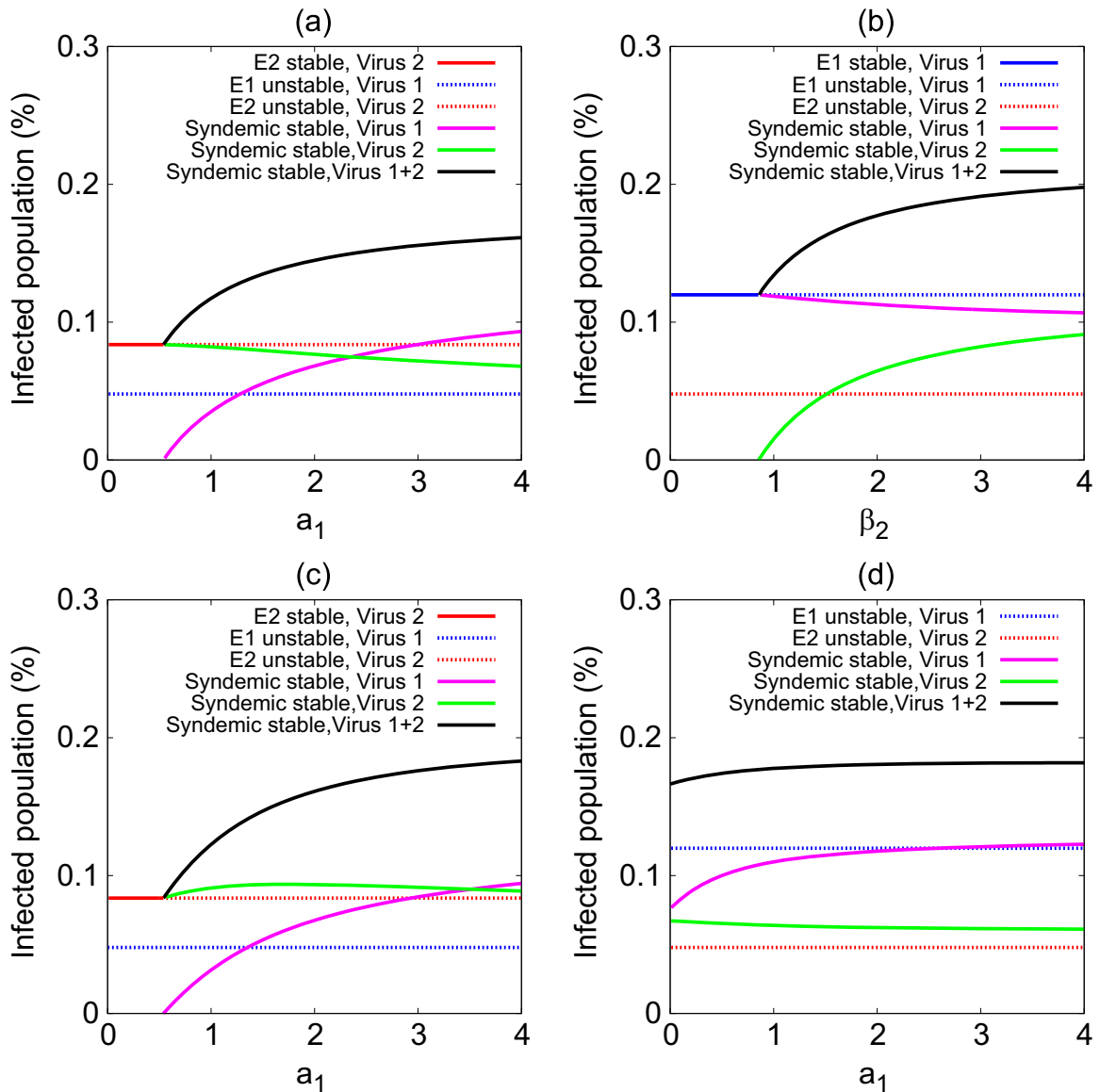
Fig. 5. Stability of endemic and syndemic steady states of the system (3) with parameter values from Table 1. Stable (unstable) steady states are indicated by solid (dotted) lines for single-virus endemic steady states E_1 (blue) and E_2 (red). The percentage of cells at the syndemic steady state is illustrated for virus 1 (magenta), virus 2 (green), and the total infected population (black). (a) $L_1 < L_2 = 2$. (b) $L_2 < L_1 = 3$. (c) $L_1 < L_2 = 2$ and $a_2 = 2$. (d) $L_2 < L_1 = 3$, $a_2 = 2$ and $\beta_1 = 1.5$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

exclusively available to each of the viruses, i.e cells that have acquired immunity to one virus may be less or more susceptible to the other virus. Our results have shown that when two viruses "antagonise" each other, i.e $a_i, \beta_i < 1$, for sufficiently high warning rates, not only can one minimise the spread of a specific virus, but the overall infection can also reduce. Hence, depending on the virulence of the two strains, one might choose to either avoid the introduction of a secondary viruses, or instead use it in order to produce the more favourable outcome.

If the two viruses are immunologically unrelated and co-infecting the same plant, they can indirectly promote each other by inadvertently making cells they can no longer infect more susceptible to the other virus. Hence, despite the fact that both viruses are effectively competing for the same resource, there is always some exclusive source of potential cells in which the infection could survive, with the potency of individual infections strongly dependent on the interaction between the two viruses. Another important result is that the syndemic steady state can potentially be stable in parameter regions where only one of the endemic steady states is feasible, implying that a secondary virus can only survive when another infection is present.

One possible extension of the work presented in this paper is to explicitly include in the model time delays associated with plant maturation time, and with delayed propagation of the RNAi signal, as has been recently done in a simpler model of immune response to a single viral infection (Neofytou et al., 2016). Another interesting phenomenon to consider is the possibility of cells being occupied by two viruses simultaneously, which would allow for a wider spectrum of interactions between the viruses and their host. This could include super-infection of individual cells, viral interference or recombination events that can give rise to additional strains.
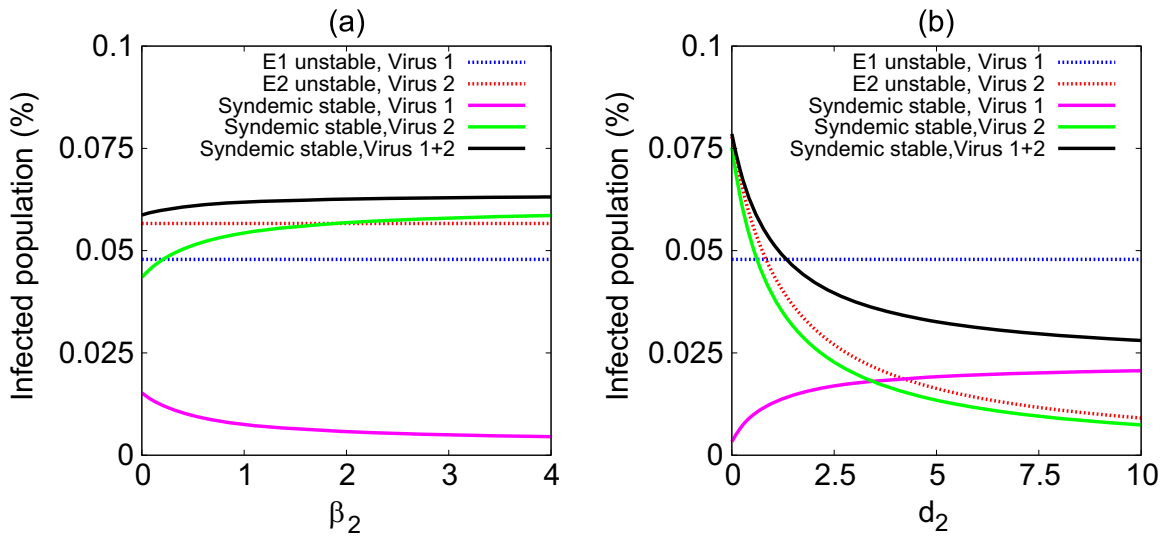
Fig. 6. Stability of endemic and syndemic steady states of the system (3) with $L_1 < L_2 = 1.6$ and the other parameter values given in Table 1. (a) $a_{1,2} = \beta_1 = 0.5$. (b) $a_{1,2} = \beta_{1,2} = 0.5$. Stable (unstable) steady states are indicated by solid (dotted) lines for single-virus endemic steady states E_1 (blue) and E_2 (red). The percentage of cells at the syndemic steady state is illustrated for virus 1 (magenta), virus 2 (green), and the total infected population (black). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
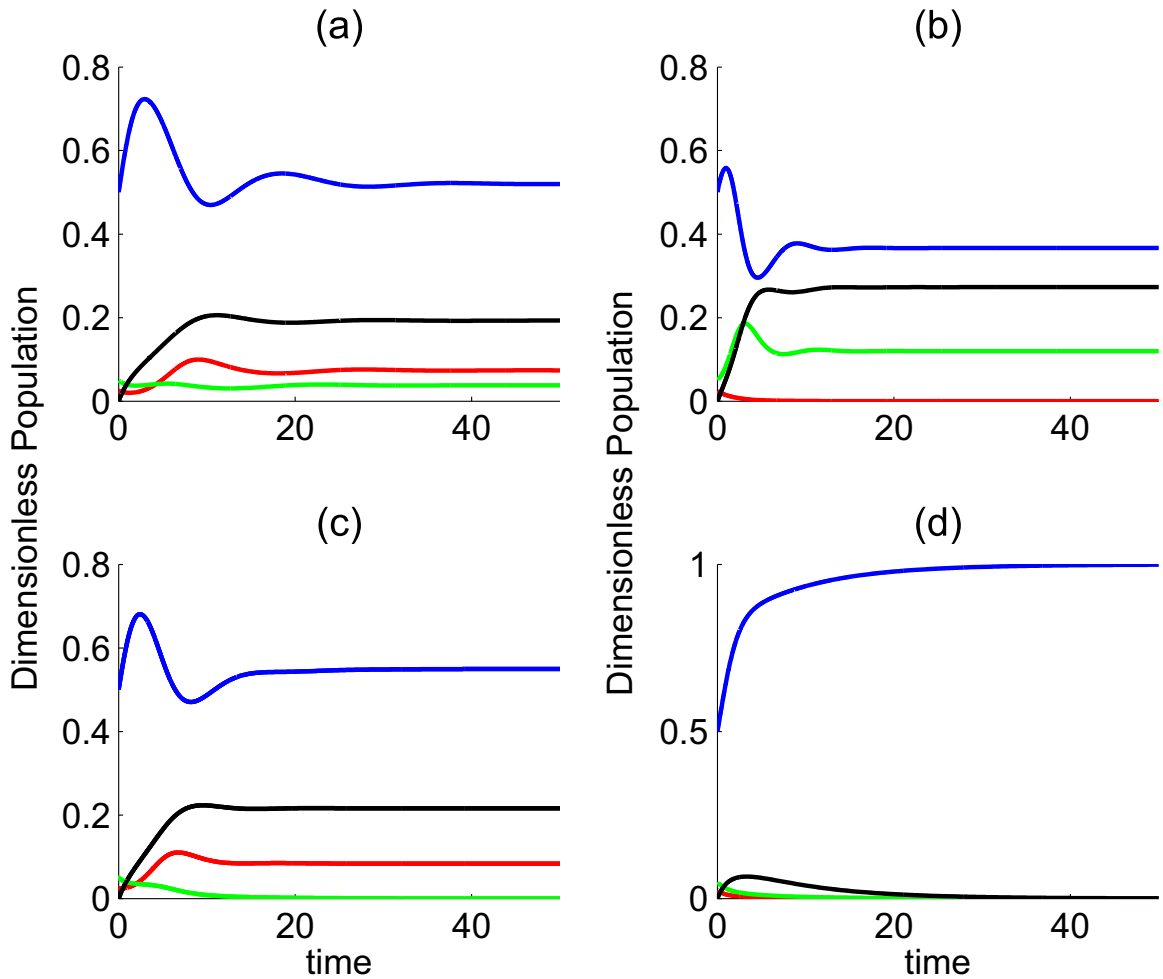

Fig. 7. Numerical simulations of the model (3) with parameter values from Table 1. Colours represent dimensionless populations of susceptible cells (blue), cells infected with the first (red) and second (green) virus, the total population of cells with immunity to one or both viruses (black). (a) Stable co-existence of viruses: $a_1 = 3$. (b) Stable single-virus state E_2: $L_1 = 1$ and $L_2 = 3$. (c) Stable single-virus state E_1: $L_1 = 2$ and $p_2 = 3$. (d) Stable disease-free steady state E_{DF}: $s_1 = 1$ and $L_2 = 1$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Acknowledgements

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