Modelling the dynamics of viral suppressors of RNA silencing

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Virus infection in plants is limited by RNA silencing. In turn, viruses can counter RNA silencing with silencing suppressors. Viral suppressors of RNA silencing have been shown to play a role in symptom development in plants. We here study four different strategies employed by silencing suppressors: small interfering RNA (siRNA) binding, double-strand RNA (dsRNA) binding and degrading or inactivating Argonaute. We study the effect of the suppressors on viral accumulation within the cell as well as its spread on a tissue with mathematical and computational models. We find that suppressors which target Argonaute are very effective in a single cell, but that targeting dsRNA or siRNA is much more effective at the tissue level. Although targeting Argonaute can be beneficial for viral spread, it can also cause hindrance in some cases owing to raised levels of siRNAs that can spread to other cells.

Keywords: viral suppressor of RNA silencing; RNA silencing; systems biology; virus–host interaction

1. INTRODUCTION

Viruses can infect all organisms and often damage their host for their own benefit of replication. Hosts have developed ways to fight off viruses, but viruses in turn developed counter-mechanisms against the host antiviral mechanisms.

RNA silencing is an evolutionary conserved mechanism that has an antiviral role in plants and insects (reviewed in [1,2]). Viral double-strand RNA (dsRNA) is cleaved into small interfering RNA (siRNA) by RNase III-like nucleases called Dicer or Dicer-like. These siRNAs are referred to as primary siRNAs. siRNAs associate with multiple proteins called the RNA-induced silencing complex (RISC). One of these proteins is an Argonaute protein (AGO), which cleaves one of the siRNA strands (the ‘passenger’ strand). This triggers unwinding of the ‘guide’ strand and activates the siRNA–RISC complex [3]. The active complex uses the siRNA as guide to target and cleave the homologous viral RNA. Secondary siRNAs can be produced through amplification pathways that use host-encoded RNA-dependent RNA (RDR) polymerase. RNA silencing can spread from cell to cell with a mobile silencing signal [4]. Recently, it has been confirmed that siRNAs are able to spread from cell to cell inhibiting spread of the virus [5].

Viruses have developed a variety of silencing suppressors (VSRs) that act on different parts of the RNA silencing pathway. A widespread strategy is the sequestering of siRNAs, therewith inhibiting the formation of active RISC. In addition, it is likely that VSR-bound siRNA is no longer able to spread from cell to cell. siRNA binding by VSRs has most probably evolved independently in different virus genera, for example, P15 in pecluviruses, pB in hordeiviruses, P21 in closteroviruses, HC-Pro in potyviruses and P19 in tombusviruses [6].

Other VSRs can bind dsRNA of all lengths, sequestering siRNAs as well as preventing dsRNA cleavage [6]. Examples are CP (or P38) of turnip crinkle virus, a carmovirus, and P14 of pothos latent virus, an aureusvirus.

Many VSRs target siRNA or dsRNA directly. There are, however, some VSRs that interact with plant Argonaute, AGO1. For example, P0 of polerovirus has been shown to be a strong suppressor of RNA silencing [7]. P0 acts either indirectly or by direct binding to Argonaute, resulting in a failure in the assembly of active RISC [8–10]. Another example is protein P1 of sweet potato mild mottle virus. Recently, it has been shown that P1 directly binds to Argonaute, inhibiting RISC activity [11].

The 2b VSR of cucumber mosaic virus also targets AGO1. However, 2b does not interfere with the ability of AGO1 to associate with siRNA. It is therefore suggested that 2b blocks the ability of AGO1 to cleave the passenger strand of the siRNA, leading to an inactive AGO1–siRNA complex [12].

Most VSRs were known as pathogenic factors, before it was discovered that they suppress RNA silencing. Because the endogenous pathways of silencing share components with antiviral silencing, it could be the...
case that VSRs interfere with host-gene regulation through microRNA (miRNA) resulting in virus-induced symptoms and developmental phenotypes. Indeed, interaction of VSRs with Argonaute affects the endogenous miRNA pathway of the host, resulting in severe abnormalities in development [8,9]. Although siRNA-binding VSRs bind only double-stranded complexes, both P21 and P19 have been shown to interfere with miRNA expression, most probably by binding the double-stranded miRNA precursors [13,14]. CP, a VSR that binds dsRNA, weakly inhibited the miRNA pathway which could explain the developmental phenotypes observed owing to CP expression [14]. These results indicate that silencing suppressors contribute to viral-induced symptoms by interfering with the miRNA pathway.

Previous models that include both viral growth and RNA silencing focused on viral growth within the cell [15] and symptom development on a tissue [16]. The effect of silencing suppressors within the cell has recently been analysed in a model by Rodrigo et al. [17]. Interestingly, they found that only a VSR that functions at the level of Dicer is able to increase the parameter region in which the virus is able to invade. They hypothesized that the beneficial effect of other VSRs may take place at the tissue level.

We here study the efficacy of the different types of VSRs and the effect they have on cellular- and tissue-level dynamics. We find that the dsRNA- and siRNA-targeting VSRs are able to increase viral spread the most at the tissue level, while the Argonaute-targeting VSRs increase intracellular viral RNA levels the most. We compare our results with the previous model studied by Rodrigo et al. [17] and with the reported observations on VSRs.

2. METHOD

We extend a previously described model of viral replication and RNA silencing that takes both intracellular- and tissue-level dynamics into account [15,16]. Important features of this model are that it accounts for realistic viral growth and plus-to-minus strand RNA ratios; for the RNA silencing primary and secondary response; for the experimentally observed skewed siRNA ratios; as well as for the spread of virions and siRNAs through a tissue [15,16]. This model consists of an intracellular model and a spatial model that connects multiple cells on a grid.

2.1. Intracellular model of antiviral RNA silencing

The intracellular dynamics are modelled by a set of differential equations describing a replicating plus-strand RNA virus and the RNA silencing pathway. We extend the previously described model with Argonaute dynamics to be able to include VSRs that target Argonaute in the analysis.

A schematic of the model is shown in figure 1, and the equations can be found in appendix A. The equations describe the dynamics of viral plus- and
minus-strand RNA, dsRNA, RdRP, virions, primary and secondary siRNAs, RISC and Argonaute. Each viral infection is initiated by the introduction of plus-strand RNA, and RNA-dependent RNA polymerase (RdRP) is translated from it. RdRP associates with plus-strand RNA to synthesize a complementary strand, and forms a dsRNA complex. The dsRNA separates into a plus and minus strand both of which can associate with RdRP again. RdRP has a higher affinity for minus-strand RNA, and multiple RdRPs can produce plus-strand RNA from a single minus-strand template. This phenomenon is known as semi-conservative synthesis of plus-strand RNA. Plus-strand RNA is packed in virions; we here use a steeply increasing Hill function to calculate the number of plus strands that is packed in virions.

Viral dsRNA is degraded into siRNAs that have a plus- or minus-strand polarity, and will target either the plus or minus strand. The ratio of siRNAs that targets the viral plus or minus strand can be very skewed: 60–98% of siRNAs are derived from the minus strand [18–20]. It has been shown that single-stranded RNA (ssRNA) can be cut by Dicer [18]. We take this into account so that the model can yield all observed siRNA ratios [15]. siRNAs can bind to Argonaute and form the siRNA–Argonaute complex. This complex can bind to other components of RISC to form the active RISC complex, which causes degradation of the target viral RNA.

The effectiveness of antiviral defence strongly depends on the secondary RNA silencing response through RDR [21,22]. RDR is even essential for restricting the spread of a VSR-defective virus [23]. In the model, the secondary response can consist of either primed or unprimed amplification. Primed amplification takes place when the siRNA binds to viral plus- or minus-strand RNA and triggers RdRP to synthesize dsRNA. Unprimed amplification is the synthesis of dsRNA from ssRNA by RdR independent of siRNA. Multiple siRNAs can be cut from the dsRNA formed by the secondary response.

We have previously shown that the unprimed secondary response is most potent in reducing viral infection [15,16]. We therefore use unprimed amplification as the default.

2.2. Spatial model of antiviral RNA silencing

To study RNA silencing and viral infection in a tissue, we use a spatial model [16]. The grid represents a number of coupled plant cells (grid size in the figures is 75 × 75). For each cell, the intracellular dynamics are calculated using the continuous model described above. A finite number of virions can move to the four neighbours. This discretization is necessary to avoid the triggering of viral replication by incomplete particles. The spread of siRNAs is implemented in the same way. Only unbound siRNAs are able to move from cell to cell. After their arrival in the new cell, virions can be unpackaged into plus-strand RNA.

2.3. Extension with viral suppressors of silencing

Equations for different VSRs are added to the intracellular model. We assume that VSRs are translated from the plus strand with the same rate as RdRP, and decay with a fixed rate. VSRs do not move from cell to cell. We study four functional groups of VSRs: siRNA binding, dsRNA binding, Argonaute decay and Argonaute inactivation. All VSRs function by binding to their target. We use a linear term to describe this process. The equations for each of the VSRs and the equations that become altered by it can be found in appendix A.

The siRNA-binding VSR associates with both primary and secondary siRNAs that target the plus or minus strand, siRNAs that associate with the VSR become inactive and are no longer able to spread from cell to cell.

The dsRNA-binding VSR binds to dsRNA produced by the virus during replication, dsRNA produced through the secondary silencing response, as well as all types of siRNA. The VSR–dsRNA complex is still functional as an intermediate in viral replication: it splits into plus- and minus-strand RNA, RdRP and suppressor. This excludes dsRNA produced by the plant in the secondary response, which does not function in the replication process. Dicer is not able to cleave siRNA from a dsRNA–suppressor complex. All siRNAs and dsRNA produced by the secondary pathway become inactive when bound to the VSR.

The VSR that targets Argonaute for decay binds to Argonaute, after which the VSR–Argonaute complex is degraded. The VSR–Argonaute complex is not able to bind siRNA.

The last type of VSR inactivates Argonaute by binding to it. The Argonaute–VSR complex binds primary and secondary siRNA as a normal Argonaute; however, the formed siRNA–Argonaute–VSR complex is not functional.

3. RESULTS AND DISCUSSION

We study the effect of four types of VSR at the cellular and tissue level. We monitor virus and siRNA levels with and without VSR, and we study virus spread.

3.1. Intracellular

Initially, we investigate the effect of the different VSRs within the cell. We infect a single cell that is capable of RNA silencing, with viral plus-strand RNA. Our default setting consists of combined double- and single-strand viral RNA cleavage by Dicer, with a secondary response of unprimed amplification (parameter values given in table 1).

In general, there are three possible outcomes of viral infection without VSRs: either the RNA silencing response is able to clear the virus from the cell, it can grow to a certain (decreased) level, or oscillations occur [15]. The outcome is determined by the growth rate of the virus and by the strength of the silencing response. The silencing strength can be varied by varying, for example, the Dicer or the RISC cleavage rate. When either is increased, viral plus-strand RNA levels decrease, until the virus is cleared from the cell (figure 2). A VSR can have two different types of effects on this behaviour. A VSR can increase the parameter region in which the virus can grow, that is, the virus...
We find that all VSRs are able to increase intracellular and investigate their effect on intracellular RNA levels. We study different binding rates for the different VSRs and siRNAs in the cell 120 h (5 days) post infection. Count the number of viral plus-strand RNAs, dsRNAs (figure 2), number of plus-strand RNA strands within the cell. VSR that targets dsRNA has the largest effect. However, we find that when RISC cleavage rate is varied, all studied VSRs increase the parameter region (figure 2b). The virus can successfully replicate (when viral replication rate and Dicer cleavage rate are varied). This result is in accordance with the findings of Rodrigo et al. [17]. They found that only a VSR that targets at the level of Dicer can increase the parameter region in which the virus can successfully replicate (when viral replication rate and Dicer cleavage rate are varied). However, we find that when RISC cleavage rate is varied, all studied VSRs increase the parameter region in which they can invade (figure 2b). But again, the VSR that targets dsRNA has the largest effect.

If the virus can expand, then all VSRs increase the number of plus-strand RNA strands within the cell (figure 2a,b).

To further study the effect of VSRs within the cell, we count the number of viral plus-strand RNAs, dsRNAs and siRNAs in the cell 120 h (5 days) post infection. We study different binding rates for the different VSRs and investigate their effect on intracellular RNA levels. We find that all VSRs are able to increase intracellular plus-strand RNA levels (figure 3a). However, not all VSRs are able to increase virus plus-strand levels to the same extent: especially, at low binding rates, the VSRs that target Argonaute are much more effective than the VSRs that bind siRNA or all dsRNA (the curves are indistinguishable in figure 3a,b).

In figure 3b,c, we show the intracellular dsRNA and siRNA levels. The shown dsRNA levels are the dsRNAs that are not bound to the VSR, so they are ‘diceable’ dsRNA. Similarly, the shown siRNA levels are the siRNAs that are not bound to VSR.

dsRNA levels are decreased by the VSR that binds dsRNA, while they are increased by the other VSRs. This is the case because increased plus-strand RNA levels result in an increase in dsRNA (figure 3b). The dsRNA-binding VSR prevents this from happening by binding dsRNA, and decreasing the number of dsRNAs that can be cleaved by Dicer.

Surprisingly, the raised dsRNA levels in the case of the other VSRs do not result in raised siRNA levels. Total siRNA levels are decreased by all VSRs, although the dsRNA-binding VSR decreases siRNA levels the most (figure 3c). Interestingly, the VSRs that target Argonaute also reduce siRNA levels, although they do not directly target siRNAs. These VSRs reduce the number of (functional) Argonautes and therefore they reduce the number of siRNAs that are not bound to VSR. Free siRNAs have a higher decay rate than the siRNAs associated with Argonaute, and the decrease in Argonaute will therefore result in a decrease in siRNA. The decrease in siRNA levels is however not as profound as in the case of the dsRNA- and siRNA-binding VSR, the.

### Table 1. Parameter values used. #mol is number of molecules.

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RNA levels. Linked, because siRNA levels also depend on plus-strand silencing response. Note that these two processes are altered siRNA levels influence the spread of the RNA virus levels will result in higher virion levels, resulting spread at the tissue level. Firstly, the increased intracellular we expect that two processes will play a role in viral suppression of RNA silencing.

In figure 4a, b, we show the results for the spatial model. To study the effect of VSRs at the tissue level, the spread of the virus in more detail, we show the number of infected cells in the tissue throughout the simulation in figure 4b. Shown is a long simulation with a high binding rate ($w = 1 \times 10^{-4}$) to compare the amount of time the different viruses take to infect the entire tissue.

In the case of unprimed amplification, viral infection without VSRs is limited to a small spot around the infected site. When a VSR is present that targets the dsRNA, the advantage for viral spread is obvious: much more cells have become infected after 5 days (figure 4a), and the entire tissue becomes quickly infected (figure 4b). The siRNA-binding VSR is less effective: for a low binding rate to siRNA, hardly more cells have become infected than the case without VSR. However, for a higher binding rate, the beneficial effect for viral spread is clear. Although the infection does not spread as fast as with the dsRNA-binding VSR, the entire tissue becomes infected in the end, even in the case of a low binding rate to siRNA (the entire tissue is infected after 1321 h).

VSRs that target Argonaute strongly increase viral plus-strand RNA levels in the intracellular case (figure 3a), but are not very effective at the tissue level (figure 4a). These VSRs only slightly increase the rate of viral spread. However, in the long run, they are able to overcome the localization of viral spread caused by RNA silencing (figure 4b).

It is striking that the Argonaute suppressors perform so well in a single cell, and so bad when a tissue is taken into account. This negative effect of Argonaute-targeting VSRs on viral spread was predicted by Baumberger et al. [8]. They hypothesized that siRNA accumulation could contribute to the observation that polerovirus spread in Nicotiana benthamiana is limited. Our results support this hypothesis. As was clear from the intracellular results, the number of free siRNAs is increased by the VSRs that target Argonaute (figure 3d). These siRNAs can spread to the neighbouring cells, bind to Argonaute and RISC and prevent spread of the virus. Also Giner et al. [11] have observed that VSR P1 cannot prevent spread of the RNA silencing response.

There are parameter regions where the Argonaute-targeting VSRs perform even worse, for example when the secondary response is absent and Dicer cleavage rate is high (figure 5, $c_2 = 15$). Without VSR, the virus spreads over the entire tissue, but with

**3.2. Tissue**

We expect that two processes will play a role in viral spread at the tissue level. Firstly, the increased intracellular virus levels will result in higher virion levels, resulting in faster spread to the neighbouring cells. Secondly, altered siRNA levels influence the spread of the RNA silencing response. Note that these two processes are linked, because siRNA levels also depend on plus-strand RNA levels.

In figure 4a, b, we show the results for the spatial model. To study the effect of VSRs at the tissue level,

maximum decrease being about one-third of siRNA levels without VSR present. siRNA levels do not decrease further when the binding rate of the VSRs to Argonaute is increased beyond the values shown in figure 3. Although the total number of siRNAs is decreased by all VSRs, the number of free, unbound siRNAs is not (figure 3d). The dsRNA- and siRNA-binding VSRs decrease the number of free siRNAs, but the Argonaute-targeting VSRs result in increased free siRNA levels. This may play a role in the spread of RNA silencing in the tissue model.

![Figure 2](http://rsif.royalsocietypublishing.org/)

**Figure 2.** The ability of VSRs to enlarge the parameter region where invasion is possible. The amount of viral plus-strand RNA for (a) increasing Dicer cleavage rate and (b) increasing RISC cleavage rate is shown. Different lines correspond to the case without VSR; with dsRNA-binding VSR; siRNA-binding VSR; the VSR that targets AGO for degradation; and the AGO-inactivating VSR. The silencing response consists of the primary and secondary pathway (unprimed amplification). Parameter values can be found in table 1.

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Argonaute-binding VSR, the infection is limited to a small spot around the infection site.

4. CONCLUSION

We studied the effect of four different types of viral-encoded VSRs of RNA silencing in a single cell and tissue model. All VSRs are able to increase intracellular virus levels, although the VSRs that target Argonaute increase them the most.

Normally, an increase in intracellular virus levels will automatically lead to higher primary siRNA levels. When silencing VSRs are present, intracellular siRNA levels can be decreased, whereas plus-strand RNA levels are increased. This is the case for all tested VSRs.

Interestingly, our results seem to contradict the findings of Rodrigo et al. [17]. They reported that only VSRs that target Dicer promote viral growth within the cell. They studied the possibility of viral expansion for different viral growth rates (translation and replication rate) and an increasing rate at which Dicer cleaves dsRNA. In our study, the VSR that targets dsRNA is comparable with their VSR that targets Dicer, because both lead to a decreased dsRNA cleavage by Dicer. When studying Dicer cleavage rate and the effect of VSRs on the possibility of viral growth, our results are in accordance with Rodrigo et al. [17]: when Dicer cleavage rate is varied, only a virus with a VSR that targets dsRNA/Dicer can enlarge the region where the virus can successfully replicate.

However, we found that all studied VSRs are able to counter a silencing response that consists of a higher RISC cleavage rate. When taking the intracellular plus-strand RNA levels into account, all VSRs are beneficial. When the virus can invade in the absence of a VSR, all VSRs can increase the number of plus-strand RNAs within the cell. We therefore conclude that all suppressors are beneficial at the cellular level.

Not all VSRs are beneficial when viral spread on a tissue is taken into account. The VSRs that target siRNA or dsRNA limit RNA silencing, and the virus reaches higher levels in more cells and spreads faster. Similar to the cellular level, the VSR that binds dsRNA is most beneficial for viral growth and spread at the tissue level. Targeting Argonaute, however, hardly increases viral spread. Nevertheless, the virus is able to overcome localization of the infection by RNA silencing so that the entire tissue becomes infected. The poor performance of the VSRs that target Argonaute is because of the increased number of free siRNAs. These siRNAs spread to cells surrounding the infected cells and limit viral spread.

Figure 3. The effect of VSRs in a single cell. Effect on (a) intracellular viral plus-strand RNA, (b) viral dsRNA, (c) total siRNA and (d) free siRNA is shown. Different lines correspond to the dsRNA-binding VSR, siRNA-binding VSR, the VSR that targets AGO for degradation; and the AGO-inactivating VSR. Shown are levels 120 h post infection with and without the presence of suppressor. Although all suppressors increase intracellular RNA levels, there are major differences in the effect on dsRNA and (free) siRNA levels. The silencing response consists of the primary and secondary pathway (unprimed amplification). Parameter values can be found in table 1.
Our results are in accordance with observations on P1—a VSR that targets Argonaute [11]. P1 cannot prevent spread of the RNA silencing response, and hardly affects siRNA production in infected plants. The suppressor, however, does increase expression of the fluorescence reporter gene. These results are very similar to our findings: we predict that VSRs that target Argonaute increase viral levels within the cell, but do not limit spread of the RNA silencing response.

The possible negative effect of the VSR on viral spread is in agreement with the observation that VSR P0 (that targets Argonaute for degradation) can hinder viral growth. It has been observed that P0 is lowly expressed and that higher expression does not increase but decreases viral growth [7]. The authors suggested that this could be the case because an increase in P0 expression automatically leads to a reduced expression of other viral proteins that are coded on the same stretch of RNA, or that it could be that P0 itself carries a negative effect for virus replication. In our model, we indeed find that this type of VSR can have a negative effect on viral spread; however higher expression of the VSR increases viral growth and spread. Our model therefore suggests that secondary effects as proposed by Pfeffer et al. [7] are indeed needed to explain the effects of P0 on viral replication and spread.

The model described here included the pathways of RNA silencing, virus growth, viral suppression of silencing and some basic cell-to-cell movement. Possible extensions of the model could involve a more detailed tissue model that includes veins and multiple connected leaves. These extensions may yield important insights to limit virus spread from tissue to tissue within the plant.

Because silencing VSRs interfere with endogenous silencing pathways, they are implicated in symptom development. Previously, we have shown that pattern formation of these symptoms on leaf tissue can be caused by viral growth and the spread of RNA silencing from cell to cell [15]. Because the number of silencing VSRs in a cell is directly correlated to the number of plus strands, we expect that symptoms develop in cells where the virus reaches high levels.

Whether or not symptoms develop by interference of VSRs with endogenous pathways or directly by viral RNA or proteins, our results indicate that these symptoms should follow the pattern generated by the interaction between viral growth and the RNA silencing response.

Figure 4. Increased spread of virus over the tissue for different VSRs. (a) Spatial distribution 120 h post infection and (b) number of infected cells over time. For each VSR, two binding rates to the target are shown. Yellow–white squares indicate cells with high numbers of viral plus-strand RNA, orange, red to black colours cells with low numbers of viral plus-strand RNA, and green squares are uninfected cells. The silencing response consists of the primary and secondary pathway (unprimed amplification). Parameter values can be found in table 1.
Figure 5. Suppressors that target Argonaute may decrease viral spread. (a) Spatial distribution 120 h post infection, parameters used: no secondary response and only dsRNA is cleaved by Dicer (a = 15). Shown are the case without VSR and with Argonaute-inactivating and Argonaute-degrading VSR (high binding rate: $w = 1 \times 10^{-1}$). (b) A graph showing the number of cells infected during the simulation shown in (a). Note that all VSRs are shown to compare their rate of spread. Default parameter values can be found in table 1.

APPENDIX A

A.1. Basic model of antiviral RNA silencing

The model consists of ordinary differential equations describing the intracellular dynamics and a spatial tissue level model.

We expand the previously described intracellular model [15] with Argonaute. Argonaute binds to siRNA and the siRNA–Argonaute complex in turn binds to other components to form the active RISC.

\[
\frac{dR}{dt} = \frac{rP}{(P + k_t)} - d_t R - \{a(1-f) P + \alpha M + \alpha d_d M \} \mathcal{F} + hD_p + hR_a + \mathcal{G}_d D_p + R_a \]  \tag{A1}

\[
\frac{dP}{dt} = -a(1-f)P \mathcal{F} + hD_p + hR_a - d_P P - \frac{vP^5}{k_P^5 + P^5} - \frac{b_P R_m P}{P + k_t} - \mathcal{G}_m P - A_P P \]  \tag{A2}

\[
\frac{dM}{dt} = -aM + hD_m \left(1 - \frac{1}{D_m}\right) - d_M - \mathcal{G}_m M - A_m M \]  \tag{A3}

\[
\frac{dV}{dt} = \frac{vP^5}{k_P^5 + P^5} - d_V V \]  \tag{A4}

\[
\frac{dD_p}{dt} = a(1-f)P \mathcal{F} - hD_p - \mathcal{G}_d D_p \]  \tag{A5}

\[
\frac{dD_m}{dt} = -\mathcal{G}_m D_m \]  \tag{A6}

\[
\frac{dS_p}{dt} = \mathcal{G}_m P + 0.5\mathcal{G}_d (D_p + D_m) - d_s S_p - b_S A_S p \]  \tag{A8}

\[
\frac{dS_m}{dt} = \mathcal{G}_m P + 0.5\mathcal{G}_d (D_p + D_m) - d_s S_m - b_S A_S m \]  \tag{A9}

\[
\frac{dR}{dt} = i - d_t R - b_t R(A_g p + A_g m) \]  \tag{A10}

\[
\frac{dR_p}{dt} = b_t R_t A_g p - d_t R_p \]  \tag{A11}

\[
\frac{dR_m}{dt} = b_t R_t A_g m - d_t R_m \]  \tag{A12}

\[
\frac{dD_e}{dt} = A_e (P + M) - \mathcal{G}_d D_e \]  \tag{A13}

\[
\frac{dS_i p}{dt} = 0.5\mathcal{G}_d D_e - d_s S_i p \]  \tag{A14}

\[
\frac{dS_i m}{dt} = 0.5\mathcal{G}_d D_e - d_s S_i m \]  \tag{A15}

\[
\frac{dA_g}{dt} = i - d_t A_g - b_t A_g(S_i p + S_i m) \]  \tag{A16}

\[
\frac{dA_g p}{dt} = b_t A_g(S_i p + S_i m) \]  \tag{A17}

\[
\frac{dA_g m}{dt} = b_t A_g(S_i m + S_i m) \]  \tag{A18}

The modelled interactions are shown in figure 1. Equations (A1)–(A7) describe viral replication and the degradation by the RNA silencing response. Equations (A8)–(A18) describe the dynamics of
proteins and RNAs of the RNA silencing response. The biological meaning of the variables is mentioned to the left of the equations. All parameter values, together with a short description, can be found in table 1. $F, G$ and $A$ are functions for the complex formation between RdRP and RNA strands, Dicer cleavage and amplification, respectively.

Viral replication is implemented as follows. Viral plus-strand RNA ($P$) is translated into RdRP ($R$), and forms virions ($V$). RdRP binds to plus-strand RNA and synthesizes dsRNA ($D_p$), which separates into plus- and minus-strand RNA ($M$). RdRP can also bind to minus-strand RNA to form dsRNA ($D_m$). More than one RdRP can bind to this double-strand complex; we refer to these as ‘active RdRPs’ ($R_a$).

The complex formation ($F$) between RdRP and RNA strands is saturated for both viral RNA and RdRP:

$$F = \frac{oR}{R + P + M + D_m + k_i}.$$  \hspace{1cm} (A19)

Viral single- or double-strand RNA is degraded into primary siRNAs by Dicer. siRNAs can target the plus strand ($S_{pm}$) or the minus strand ($S_{pm}$). siRNAs cleaved from double-strand RNA will target plus-strand or minus-strand RNA with a 1 : 1 ratio. Secondary siRNA ($S_{mp}$ and $S_{mp}$) is cleaved from dsRNA produced by host-encoded RDR ($D$).

The Dicer cleavage function is saturated for $D_p$, $D_m$, $P$ and $M$. The Dicer cleavage functions, one for cleaving dsRNA and one for ssRNA, are saturated according to the ratio between single- and double-strand RNA cleavage ($q$) by Dicer:

$$G_d = \frac{(1 - q) c_d D}{(1-q)(D_p + D_m + D_c) + q(P + M) + k_i}.$$  \hspace{1cm} (A20)

and

$$G_{p,m} = \frac{q c_d D}{(1-q)(D_p + D_m + D_c) + q(P + M) + k_i}.$$  \hspace{1cm} (A21)

Free Argonaute ($Ag$) associates with primary and secondary siRNA. $Ag_p$ and $Ag_m$ represent siRNA–Argonaute complexes with either a plus- or minus-strand siRNA. These complexes can associate with proteins to form the active RISC ($R_p$ and $R_m$). RISC degrades the viral plus- or minus-strand RNA.

The unprimed amplification is described by

$$A_m = \frac{a_m}{(P + M + k_i)}.$$  \hspace{1cm} (A22)

The above equations are calculated for each cell on the tissue. Virions and siRNAs can move to the four neighbours of a cell. In each cell, virions are unpacked into plus-strand RNA. To avoid triggering of viral growth or RNA silencing by incomplete particles, we move virions and siRNAs with a particle-based system. A fraction of virions and siRNAs is evenly distributed among the neighbours. Excess virions are distributed randomly, and when the number of moving particles is smaller than one, we draw a number to decide if a particle moves to a random neighbour.

A.2. siRNA binding

We add an equation for the VSR ($S$) to the intracellular model.

The VSR is translated from the plus strand with maximum rate $r$ and saturation constant $k_i$, and decays with rate $d_s$. The VSR associates with primary and secondary siRNAs with rate $w$. The association terms $wS_{mp}, wS_{mp}$, $wS_{mp}$, $wS_{mp}$ and $wS_{mp}$ are subtracted from the siRNA equations. Those siRNAs are no longer able to associate with Argonaute, and cannot spread from cell to cell.

We show the altered equations with the new terms in bold print:

VSR

$$\frac{dS}{dt} = \frac{rP}{(P + k_i)} - d_sS - w(S_{mp} + S_{mp}) + S_{mp} + S_{mp}$$  \hspace{1cm} (A23)

$+$ siRNA

$$\frac{dS_{mp}}{dt} = g_{p,m}P + 0.5g_d(D_p + D_m) - d_sS_{mp} - b_0AgS_{mp} - wS_{mp}$$  \hspace{1cm} (A24)

$-$ siRNA

$$\frac{dS_{mp}}{dt} = g_{p,m}M + 0.5g_d(D_p + D_m) - d_sS_{mp} - b_0AgS_{mp} - wS_{mp}$$  \hspace{1cm} (A25)

sec. + siRNA

$$\frac{dS_{mp}}{dt} = 0.5g_dD_p - d_sS_{mp} - b_0AgS_{mp} - wS_{mp}$$  \hspace{1cm} (A26)

sec. $-$ siRNA

$$\frac{dS_{mp}}{dt} = 0.5g_dD_m - d_sS_{mp} - b_0AgS_{mp} - wS_{mp}.$$  \hspace{1cm} (A27)

A.3. dsRNA binding

Because dsRNA is really the core variable of both viral replication and RNA silencing, this VSR alters almost all equations, and adds four variables to the model. The new variables are $S$: VSR protein; $S_{mp}$: VSR associated with dsRNA from the plus strand; $S_{mp}$: VSR associated with dsRNA from the minus strand; and $S_{mp}$, active RdRPs on $S_{mp}$. VSR protein is translated from plus-strand RNA with maximum rate $r$ and saturation constant $k_i$, and has a decay rate $d_s$. VSR associates with $S_{mp}$, $S_{mp}$, $S_{mp}$, $S_{mp}$, $D_m$, $D_p$ and $D_c$, with rate $w$. The association terms are subtracted from the corresponding equations. We assume that the VSR–siRNA complex and the VSR–D complex remain inactive after association. The complexes of $D_p$ and $D_m$ with VSR, $S_{mp}$ and $S_{mp}$ dissociate in plus- and minus-strand RNA, RdRP and VSR with rate $h$. To maintain semi-conservative replication of plus-strand RNA from the minus strand when VSR associates with $S_{mp}$, the mean number of active RdRPs is removed from the normal $R_a$ pool and added to the $S_{mp}$ pool. Because viral replication now functions also through the VSR–dsRNA complexes,
The altered equations are

\[
\begin{align*}
\frac{dS}{dt} &= \frac{rP}{(P + k_r)} - d_S - w(D_p + D_m + D_e)S \\
&\quad - w(S_{im} + S_{im} + S_{ip} + S_{ip})S + hS_{dp} \\
&\quad + hS_{dm}(1 - \frac{1}{S_{dm}})^{(s_i-r_{im})} \\
\frac{dR}{dt} &= \frac{rP}{(P + k_r)} - d_R - \{o(1-f)P + ofM \\
&\quad + o_0 D_m + o_0 S_{dm}\}R + hD_p + hS_{dp} \\
&\quad + hR_a + hS_{ra} + G_i(D_p + R_a) \\
\frac{dP}{dt} &= -o(1-f)P F + hD_p + hS_{dp} + hR_a \\
&\quad + hS_{ra} - dP - \frac{wP^5}{k_{i}^5 + P^5} - \frac{b_2 R_m P}{P + k_i} \\
&\quad - G_{p,m}P - A_u P \\
\frac{dM}{dt} &= -oM F + hD_p + hS_{dp} \\
&\quad + hD_m(1 - \frac{1}{D_m})^{(R_i - D_m)} \\
&\quad + hS_{dm}(1 - \frac{1}{S_{dm}})^{(s_i-r_{im})} \\
&\quad - dM - \frac{b_2 R_m M}{M + k_i} - G_{p,m} M - A_u M \\
\frac{dD_p}{dt} &= o(1-f)P F - hD_p - G_i D_p \\
&\quad - wD_p S \\
\frac{dD_{dm}}{dt} &= wD_m S \\
&\quad - hS_{dm}(1 - \frac{1}{S_{dm}})^{(s_i-r_{im})} \\
\frac{dR_a}{dt} &= ofM F + o_0 D_m F - hR_a \\
&\quad - G_i R_a - wR_a S \\
\frac{dS_{ra}}{dt} &= wR_a S + o_0 S_{dm} F - hS_{ra}
\end{align*}
\]
sec. $+$siRNA  \[ \frac{dS_{\text{sp}}}{dt} = 0.5G_{d}D_{v} - d_{\text{sp}}S_{\text{sp}} - b_{0}\text{Ag}S_{\text{sp}} \]
- $b_{0}\text{Ag}S_{\text{sp}}$ (A.51)

sec. $-$siRNA  \[ \frac{dS_{\text{sm}}}{dt} = 0.5G_{d}D_{v} - d_{\text{sm}}S_{\text{sm}} \]
- $b_{0}\text{Ag}S_{\text{sm}}$ - $b_{0}\text{Ag}S_{\text{sm}}$. (A.52)
siRNAs that associate with inactive Argonaute are removed from the free siRNA pool.

A.6 Parameters

All default parameter values can be found in Table 1. If another value is used, it is explicitly mentioned in the figure captions. Previously, we have extensively studied the parameters of both the intracellular and the spatial models [15,16]. Many parameters have a similar effect on the dynamics, because there are only a few outcomes of viral infection at the cellular level. Either the virus successfully replicates or it cannot expand. In this study, we explore the binding rate of the VSRs to their target ($w$), and the differences between the different mechanisms of suppression. Additionally, we use Dicer and RISC cleavage rate to study the ability of the VSRs to counter by varying strengths of the silencing response. We therefore keep the other parameters constant. They were chosen within the ranges of the previous studies, and such that the virus is not able to expand over the entire tissue (otherwise we would see no effect of the suppressor). The only new parameters are $b_{0}$, which represents the rate of siRNA–Argonaute complex formation, and $w$, the binding rate of the VSRs to their target. We chose $b_{0}$ such that the outcome of our simulations was the same as without the explicit modelling of Argonaute. Because no value for $w$ is known from the literature, we varied $w$ from 0 to a value that showed a clear effect of the suppressors, in this case 0.0001. Increasing $w$ beyond 0.0001 results in faster spread over the tissue for all VSRs. Rate of spread over the tissue is always largest for the VSR that targets dsRNA, then siRNA, Argonaute inactivation and the slowest is Argonaute decay.

REFERENCES


