The games plant viruses play

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Mixed virus infections in plants are common in nature. The outcome of such virus-virus interactions ranges from cooperation and coexistence (synergism) to mutual exclusion (antagonism). A priori, the outcome of mixed infections is hard to predict. To date, the analyses of plant virus mixed infections were limited to reports of emerging symptoms and/or to qualitative, at best quantitative, descriptions of the accumulation of both viruses. Here, we show that evolutionary game theory provides an adequate theoretical framework to analyze mixed viral infections and to predict the long-term evolution of the mixed populations.

Highlihgts:

- The outcome of mixed virus plant infections is complex and hard to predict
- Game theory provides tools to study interactions between viruses
- Mutual cooperation is the most commonly game played by plant viruses
- Molecular biology of viruses may explain how certain games are played

Introduction

An interesting phenomenon of plant viral disease, which is not so common among animal viruses, is synergism during coinfection [1**]. Coinfection by two distinct viruses or by two strains of the same virus results in complex interactions with unpredictable disease phenotypes [1**]. In general, antagonistic interactions lead to mutual exclusion and, eventually, to cross-protection. By contrast, synergism enhances fitness components of at least one of the members of the viral complex and the effect on the host becomes greater than the sum of the individual effects. Mixed infections can also modify viral traits such as host range [2-5], transmission rate [6,7], cellular tropism [8,9], titer [3,10*,11], and even the biology and preference of vectors [12]. Umbraviruses and their luteovirus helpers provide particularly suggestive examples of synergism, illustrating the degree of dependence that can be achieved among pairs of viruses. Umbraviruses such as *Groundnut rosette virus* (GRV) do not encode for a coat protein and use the capsids of its luteovirus helper *Groundnut rosette assistor virus* (GRAV) for aphid transmission [13]. In this particular case, the situation is even more

complex since encapsidation of GRV in the GRAV capsid proteins requires of the intervention of a GRV satellite RNA that co-encapsidates [13].

In mixed infections, each viral population changes the environment and becomes part of the fitness landscape of the co-infecting virus population. Therefore, in mixed infections the success of any virus depends not only on its adaptation to the host, but also on how its behavior interacts with that of others. So the fitness of an individual virus cannot be measured in isolation; rather it has to be evaluated in the context of the community in which it lives. This opens the door to a natural game-theoretic analogy: genetically-determined characteristics and behaviors of a virus are like its strategy in a game, its fitness is like its payoff, and this payoff depends on the strategies of the viruses with which it interacts. Biological games can be conveniently modeled and analyzed using the mathematical framework provided by game theory [14,15**]. The players have to choose between strategies whose payoff depends on their rivals' strategies [15**,16]. Cooperation and defection are the two strategies that are usually at the heart of every social dilemma. While cooperative individuals contribute to the collective welfare at a cost, defectors choose not to [15**-17].

The payoff matrix

In the simplest situation, two players can chose between two strategies, cooperation or defection. Viral cooperation and defection can be defined as, respectively, the manufacturing and sequestering of diffusible (shared) intracellular products (Figure 1). The 2×2 payoff matrix shown in Table 1 is the fundamental tool to analyze and predict the outcome of the contest. Each player has a different fitness depending on the frequency of competitors in the population. The entries denote the payoff for the row player. If player A decides to cooperate, its fitness would depend on player B's response. If B cooperates, then both players receive a fitness reward *R* for their mutual cooperation. However, if B decides to defect, A suffers a reduction in fitness *S* (the sucker's payoff). Lets turn now to the situation in which A decides to defect. If B still cooperates, then A gets an additional fitness bonus *T* (the temptation of defect). Finally, if both players decide to defect, they both suffer a fitness punishment *P*.

In a well-mixed population (*i.e.*, no spatial structure exists in the system and thus all encounters are equally likely to happen), there are four possible outcomes, depending

on the relative values of the four elements of the payoff matrix [16,17]. (*i*) If $R > T$ and $S > P$, then no conflict exists and mutual cooperation (MC) is the dominant strategy. Indeed, this solution provides a single stable equilibrium in which both populations coexist. (*ii*) If parameters satisfy the condition $R < T$ and $S < P$, then mutual defection is the dominant strategy, which also corresponds to a stable equilibrium in which, in the long term, only one of the populations persists. This game corresponds to the famous Prisoner's Dilemma (PD). (*iii*) If $R > T$ and $S < P$, then both contenders will benefit when acquiring the same strategy (mutual cooperation or mutual defection), but will suffer punishment when choosing a strategy different from that of their counterpart. This game is known as the Stag-Hunt (SH) and gives rise to two symmetric non-stable equilibria, thus representing a potential problem as populations may get trapped in the suboptimal mutual defection equilibrium. Finally, (*iv*) if parameter values are such that $R < T$ and $S > P$, we are in the domain of the Hawk-Dove game (HD). In this game cooperation is better only when the other player defects and vice versa, defection is better when the opponent cooperates. The stable equilibrium for this game is to adopt a strategy different from that of the other player.

Last, another concept to keep in mind is that of an evolutionary stable strategy (ESS). Maynard Smith [19] defined a population as evolutionary stable if it is resistant to invasion by a small group of individuals playing a different game. Put in other words: if a virus were genetically determined to cooperate, would it persist in the presence of another virus that is defecting? The mathematical conditions for ESS are that $R > T$ or that $R = T$ and $S > P$. Thus cooperation is an ESS in MC and SH games.

Common games among plant viruses

Available data are scarce to properly address whether plant viruses may prefer one strategy to another. Yet, Table 2 shows the results from the analyses of payoff matrices constructed for a set of 25 viruses, from 10 genera (including both DNA and RNA genomes), in 18 pairwise combinations. Regardless the nature of the viruses involved in the pair, MC is the most commonly played game. This game represents the case of perfect synergism in which both competitors benefit from each other. Since MC is an ESS, two viruses involved in this game will coexist in the infected plant for long periods of time. The second most commonly played game is HD. According to this game, both viruses compete for a limiting resource. If one competitor is better using a

given resource than the other, then it gets full benefit; but if both competitors are equally fit, they negatively affect each other. Intuitively, a *hawk* will do very well in a population of *doves* – but in a population of all *hawks*, a *dove* will actually do better by staying out of the way while the *hawks* fight with each other. HD is not an ESS, meaning that two viruses cannot persist together for long, as one will ultimately outcompete the other.

Table 2 suggests some other interesting things that need to be explored in the future. First, the game chosen by a pair of viruses may depend on the host. Indeed, ZYMV and CMV play MC in most cases but switch to SH in a particular host. Second, the developmental stage of the plant may also influence the game played. In young leaves, where cell division takes place and photoassimilates are being imported, competition between SPFMV and SPCSV is strong for infecting the new cells and the interaction is ruled by an SH game. By contrast, in mature leaves, where cell division is not so important and photoassimilates are being actively exported, promoting systemic virus movement out of the leaf, these two viruses are playing MC. Finally, given a viral genus, the probability of choosing a particular strategy varies depending on the competitor. For instance, potyviruses show significant heterogeneity in their responses ($\chi^2 = 20.250$, 6 d.f., $P < 0.001$): MC is the most common against cucumoviruses but HD is the most common against criniviruses.

Games are grounded in molecular interactions

One of the best-studied mechanisms of synergism is via the blockage of the RNA silencing antiviral response by viral suppressors of RNA silencing (VSR) [34-37**] (Figure 1). For example, potyvirus SPFMV and crinivirus SPCSV are mostly involved in HD games, which is not ESS (Table 1). It has been long established that potyviruses may enhance other viruses' replication due to their VSR HC-Pro. HC-Pro is a cytoplasmic protein that prevents the assembly of siRNAs into the active RISC by binding to and sequestering duplex siRNAs [38]. However, SPCSV does not need to use HC-Pro since it encodes for a dsRNA-specific class 1 RNA endoribonuclease III (RNase3) that suppresses RNAi in an endonuclease activity-dependent manner [39]. RNase3 cleaves siRNAs to products of 14 bp that are inactive in RNAi. Therefore, both viruses compete in equal conditions to exploit the cell until one displaces the other. It is impossible to predict who the winner will be. A different example is coinfection

between potyviruses and the cucumovirus CMV [25,40, 41]. In general, potyviruses and CMV are involved in a MC game, which is an ESS (Table 1). CMV VSR 2b accumulates in the nucleus and cytoplasm of plant cells and inhibits intercellular silencing spread and may also play a role in enhancing translation by suppressing translational repression caused by siRNAs. Therefore, cooperation may arise from HC-Pro assisting in CMV replication at the single-cell level whereas 2b may facilitate systemic movement of potyviruses by blocking the spread of potyvirus-specific siRNAs. In this situation, both viruses can coexist indefinitely.

Synergism does not always depend on the suppression of RNA silencing. Other mechanisms exist. For example, begomovirus protein C2 expression reactivates cell cycle, thus promoting the replication of other coinfecting begomovirus but not of RNA viruses [9]. As an additional example, the potyvirus movement protein P3N-PIPO facilitates the systemic spread of the potexvirus *White clover mosaic virus* without enhancing virus accumulation per cell [42] (Figure 1). In some instances, synergism may result for more complex reasons than the action of a few viral proteins. For example, coinfection of *Nicotiana benthamiana* by PVY and the potexvirus *Potato virus X* results in aggravation of symptoms as a consequence of the altered expression of many genes that were not altered by any of the single infections [43]. These genes were scattered among several metabolic pathways, confirming that host response to virus synergism includes a coordinated rearrangement of a wide array of cellular processes rather than a simple induction of genes involved in stress response.

Conclusions

Simple viruses exhibit surprisingly complex behaviors during competition. In each case, evolutionary game theory sheds light on the exact mechanism by which interaction takes place. From an academic perspective, more systematic data will allow to explore the reproducibility of the games, to analyze whether certain viral genera have a tendency to play a given game, and to explore the influence of host species, of other environmental factor, and of plant developmental stages on the games played by viral contenders. Experiments must be designed keeping in mind the sort of information needed to construct payoff matrices. And a further step will be to analyze multi-player (> 2) games.

From a more practical perspective, evolutionary game theory may contribute to better understand the foundations of cross-protection. Cross-protection describes the phenomenon by which infection with a mild strain protects against subsequent infection from a severe one of a closely related virus [1,44]. Genetically modified plants do not perform as well as expected against viral infections. Therefore, the usage of co-infecting viruses to cross-protect seems a reasonable strategy worth exploring.

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Table 1

The payoff matrix for the interaction between two players

		Type of adversary (B)	
		Cooperator	Defector
Player who wins the payoff (A)	Cooperator	*R*	*S*
	Defector	*T*	*P*

The standard scaled parameterization entails designing $R = 1$ and $P = 0$ as fixed [18].

Table 2

List of studies on mixed infections used in this review. See Supplementary File 1 for experimental details and values of the payoff matrices.

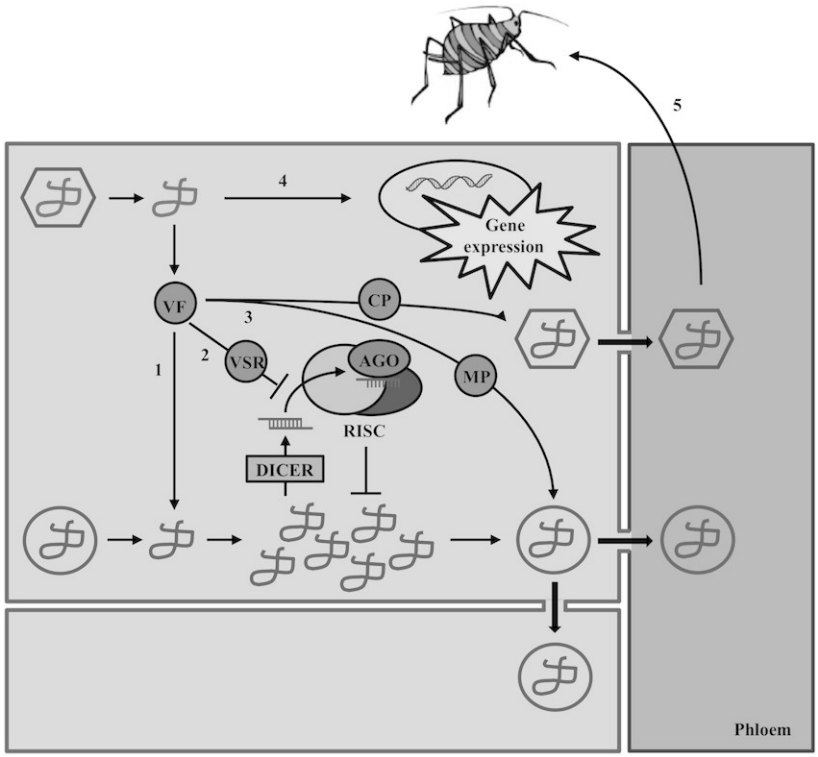
Reference	Player A	Player B	Game[1]
[7]	ToCV (crinivirus)	TICV (crinivirus)	HD (in *Physalis wrightii*) or MC (in *Nicotiana benthamiana*)
[10]	TuMV (potyvirus)	CaMV (caulimovirus)	MC
[11]	TMV (tobamovirus)	HLSV (tobamovirus)	MC
[12]	PVY (potyvirus)	PLRV (polerovirus)	MC
[20]	ToYSV (begomovirus)	ToRMV (begomovirus)	HD (both in *Solanum lycopersicum* and *N. benthamiana*)
[21]	ZYMV (potyvirus)	CMV (cucumovirus)	MC
[22]	ToLCPalV (begomovirus)	ToLCNDV (begomovirus)	HD (for DNA A) or MC (for DNA B)
[23]	SPFMV-Nam1 (potyvirus)	SPCSV (crinivirus)	HD (in young leaves) or MC (in mature leaves)
[24]	SPFMV-RC (potyvirus)	SPCSV (crinivirus)	MC
	SPFMV-C (potyvirus)	SPCSV (crinivirus)	HD
	SPFMV-95.6 (potyvirus)	SPCSV (crinivirus)	HD
	IVMV (potyvirus)	SPCSV (crinivirus)	HD
	SPVG (potyvirus)	SPCSV (crinivirus)	HD
[25]	PVY (potyvirus)	CMV-Fny (cucumovirus)	HD

[26]	PepMoV (potyvirus)	CMV-Fny (cucumovirus)	HD
[27]	TMV (tobamovirus)	AbMV (begomovirus)	MC
[28]	PHYVV (begomovirus)	PepGMV (begomovirus)	SH
[29]	WSMV (tritimovirus)	MCMV (tombusvirus)	MC
[30]	TuMV (potyvirus)	CMV-Y (cucumovirus)	MC or HD (data do not allow to discriminate among these games)
[31]	WSMV (tritimovirus)	TriMV (poacevirus)	MC
[32]	ZYMV (potyvirus)	CMV-Fny (cucumovirus)	MC
	ZYMV (potyvirus)	CMV-LS (cucumovirus)	MC
[33]	ZYMV (potyvirus)	CMV-Fny (cucumovirus)	SH (in *Cucumis sativus*) or MC (in *Lagenaria siceraria*)

ToCV, *Tomato chlorosis virus*; TICV, *Tomato infectious chlorosis virus*; TuMV, *Turnip mosaic virus*; CaMV, *Cauliflower mosaic virus*; TMV, *Tobacco mosaic virus*; HLSV, *Hibiscus latent Singapore virus*; PVY, *Potato virus Y*; PLRV, *Potato leafroll virus*; ToYSV, *Tomato yellow spot virus*; ToRMV, *Tomato rugose mosaic virus*; ZYMV, *Zucchini yellow mosaic virus*; CMV, *Cucumber mosaic virus*; ToLVPalV, *Tomato leaf curl New Delhi virus*; ToLCNDV, *Tomato leaf curl Palampur virus*; SPFMV, *Sweet potato feathery mottle virus*; SPCSV, *Sweet potato chlorotic stunt virus*; IVMV, *Ipomoea vein mosaic virus*; SPVG, *Sweet potato virus G*; PepMoV, *Pepper mottle virus*; AbMV, *Abutilion mosaic virus*; PHYVV, *Pepper huasteco yellow vein virus*; PepGMV, *Pepper golden mosaic virus*; WSMV, *Wheat streak mosaic virus*; MCMV, *Maize chlorotic mottle virus*; TriMV, *Triticum mosaic virus*.

[1]Not all games are equally represented in the sample ($\chi^2 = 20.533$, 3 d.f., $P < 0.001$). There is a significant enrichment in pairs of viruses playing MC and HD, whereas SH and PD are significantly underrepresented.

Figure 1. Molecular pathways in which a virus (in red) may impact the infec
of a second co-infecting virus (in blue). Mesophyll cells are depicted in g
phloem tissue in brown. Viruses are represented by their genome and the cap
uncoating, virus replicates and accumulates within the cell, forming new vira
that may move from cell to cell via plasmodesmata until being loaded into tl
for systemic long-distance movement. The major processes that may be aff
consequence of viral synergism are indicated with numbers: 1, viral replicatio
silencing; 3, virus movement, either cell-to-cell or long-distance through the p
host gene expression; 5, virus transmission by vectors. VF refers to vi
mediating the synergistic effect: VSR, viral suppressor of RNA silenc
movement protein; CP, coat protein. Components of the plant RNA
machinery are also shown: DICER, endonuclease responsible for generat
interfering RNAs (siRNAs) from dsRNA; siRNAs are recognized by argonaut
(AGO) and incorporated into the RNA-induced silencing complex (RISC), v
eventually target the complementary RNA sequence. A virus may
accumulation of another co-existing virus by directly or indirectly altering its r
rate (1), or interfering with inhibitory plant RNA silencing mechanisms by v
suppressor activity (2). It may also complement deficient short- or lon
movement of the accompanying virus, (3), differentially modify host gene e
(4), or facilitate horizontal transmission by vectors, *e.g.* by heteroencapsic
While mostly cooperative, and shown here as independent for the p
simplification, these interactions may be antagonistic in some cases and may
interconnected.


5
4
Gene
expression
VF
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3
2
VSR
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MP
1
RISC
DICER
Phloem