When Viruses Play Team Sports: Mixed Infections in Plants

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ABSTRACT

The pathological importance of mixed viral infections in plants might be underestimated except for a few well-characterized synergistic combinations in certain crops. Considering that the host ranges of many viruses often overlap and that most plant species can be infected by several unrelated viruses, it is not surprising to find more than one virus simultaneously in the same plant. Furthermore, dispersal of the majority of plant viruses relies on efficient transmission mechanisms mediated by vector organisms, mainly but not exclusively insects, which can contribute to the occurrence of multiple infections in the same plant. Recent work using different experimental approaches has shown that mixed viral infections can be remarkably frequent, up to the point that they could be considered the rule more than the exception. The purpose of this review is to describe the impact of multiple infections not only on the participating viruses themselves but also on their vectors and on the common host. From this standpoint, mixed infections arise as complex events that involve several cross-interacting players, and they consequently require a more general perspective than the analysis of single-virus/single-host approaches for a full understanding of their relevance.

Keywords: disease control and pest management, virology

The diversity of viruses is extraordinary, as virtually all kinds of cellular life forms are susceptible to being parasitized by many different viruses; plants are no exception (Wolf et al. 2018). Plant viruses are grouped in numerous families (about 30) and genera (>145) with extraordinary heterogeneity, which makes them one of the most abundant groups of pathogens responsible for a large number of all known plant diseases. For example, nearly 1,500 species are listed in the International Committee on Virus Taxonomy (2018) report. Our knowledge of the virosphere is currently facing a major challenge to classify and accommodate many recently discovered viruses (Koonin and Dolja 2018; Zhang et al. 2019). One of the reasons explaining why this enormous diversity was somehow overlooked until now comes from the fact

that attention was mostly focused on agents of diseases and, in the case of plants, only viruses causing economic losses in crops and ornamentally valuable species have been deeply studied for decades. A particularly interesting case of this tendency to bias our knowledge is the frequent existence of mixed infections. This common situation was likely underestimated in available studies, with not as many reported examples, despite the fact that many plant viruses are generalists and thus able to infect different plants, including both crops and wild plants (Alexander et al. 2014; Elena et al. 2009). Furthermore, many vectors of plant viruses are polyphagous or are organisms associated with multiple hosts, capable of transmitting more than one virus to the same plant (Syller 2014). All of these conditions argue in favor of a more frequent presence of multiple viruses in plants; indeed, metagenomics approaches using high-throughput next-generation sequencing techniques have begun to reveal the real extent of mixed infections between known and occasionally unknown viruses in the virome in plant hosts, including wild plants and crops (Al Rwahnih et al. 2009; Mascia and Gallitelli 2016; Roossinck et al. 2010, 2015). Until relatively recently, symptomless wild plants were rarely studied, although work conducted on weeds and wild plants growing within or around crop fields often revealed the presence of multiple viruses. In addition, there are many reports deriving from surveys in crop plants that showed multiple viral infections in the examined samples. Unfortunately, these types of descriptive studies are frequently not complemented with work on

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the interactions occurring between the viruses involved, unless they were responsible for epidemics causing major crop losses. In addition, the technology to conduct global analysis was not broadly available until recently. Nevertheless, the new concept of the virome is finally gaining interest in the research community (including funding agencies) and logically, plant virologists are intensifying their efforts to describe “what is out there.” Consequently, we believe that there will be more frequent detections of mixed viral infections in the coming years. Therefore, we think it is timely to review what it is known about mixed viral infections, acknowledging that they can be quite complex and that their establishment relies not only on the properties of each one of the actors involved (viruses, common host, and vector or vectors) but also on the relationship and interactions between them. Moreover, other layers of complexity can be added to multiple viral infections, such as the presence of natural enemies of the vectors or the influence of nonviral pathogens in the host plant. However, those aspects are explored elsewhere and are not addressed in this review (Dáder et al. 2012; Jeger et al. 2011a, b; Prager et al. 2015). Similarly, the importance of multipathogenic attacks (Abdullah et al. 2017) and even the combination of biotic and abiotic stresses in more holistic approaches (Saijo and Loo 2019) has come to researchers’ attention. Nevertheless, we will concentrate here on the combination of heterologous viruses, although it is important to keep in mind that these cases are only a subset of many other putative relationships between the numerous sources of stress that plants must face.

Some mixed viral infections have been the cause of important diseases in crops worldwide, as they induce a decline in plant vigor and productivity. Logically, these cases have been studied in depth. A first example is corn or maize lethal necrosis (MLN), which affects one of the main crop species in the world and causes economically devastating losses. MLN results from the combination of the machlomovirus maize chlorotic mottle virus and different members of the family *Potyviridae* (Mahuku et al. 2015; Redinbaugh and Stewart 2018; Scheets 1998; Stewart et al. 2017; Uyemoto et al. 1980).

Sweet potato viral disease (SPVD) is another good example of the consequences of a well-characterized mixed viral infection (Clark et al. 2012). Sweet potato is considered a crop essential for food security in extended areas, as it is a basic dietary component for large populations in China and Africa. One of the peculiarities of sweet potato compared with most other crops is attributable to its rapid multiplication by vegetative propagation of cuttings, a rather beneficial agronomic trait that implies a high risk of accumulating pathogens, particularly viruses. Likely most marketable sweet potatoes are infected simultaneously by several viruses of the remarkably large list of >30 different viruses that can infect this plant. SPVD results from the synergistic interaction of isolates of the crinivirus *Sweet potato chlorotic stunt virus* (SPCSV) and the potyvirus *Sweet potato feathery mottle virus* (SPFMV) and can cause losses up to 90% of the crop yield (Karyeija et al. 2000). Another well-known viral combination in the same crop is sweet potato severe mosaic disease, caused again by synergism between SPCSV and the ipomovirus sweet potato mild mottle virus (SPMMV) (Mukasa et al. 2003; Tugume et al. 2005).

Cassava is also another important staple food crop with a high incidence of multiple viral infections. As in the case of sweet potato, the vegetative propagation is often perpetuating infections by many viruses (Jacobson et al. 2018; Legg et al. 2015; Rey and Vanderschuren 2017; Zinga et al. 2013). Research is revealing some particularly complex viral combinations on this host (Abarshi et al. 2012; Carvajal-Yepes et al. 2014; Reddy et al. 2012).

Another example of a crop with important problems caused by multiple viruses is grapevine. In this case, the cultivation method relying on grafting and propagation of clonal varieties also can favor mixed infections. Currently, approximately 70 viruses and

virus-like agents have been documented in different grapevine cultivars worldwide (Martelli 2014). Grapevine leafroll disease is an extreme example of a multiple viral infection, as it is a complex disease caused by the association of up to 11 grapevine leafroll-associated viruses (Naidu et al. 2014, 2015).

In addition, some subviral agents or viral-associated replicons can be also considered examples of mixed infections. For instance, the alphasatellites associated with geminiviruses and nanoviruses (Briddon et al. 2018) were found to reduce infectivity in some cases and to either reduce or exacerbate symptoms and even to affect vector transmission (Mar et al. 2017). Another example are the small RNA replicons that often associate with poleroviruses, like the one associated with isolate ST9 of beet western yellows virus: this small RNA is replication competent but absolutely dependent on the helper virus for movement and aphid transmission (Falk and Duffus 1984; Falk et al. 1989). Interestingly, its presence stimulates the replication of the helper polerovirus and confers symptom severity (Passmore et al. 1993; Sanger et al. 1994). Although we will focus mainly on the interactions between viruses, we will also consider other associated agents in some cases.

All of the examples mentioned, together with the new knowledge that metagenomics approaches are bringing to the surface, reveal that mixed infections occur in a high frequency. However, a vast number of research efforts in plant virology still deal only with single infections. This might correspond to the difficulties and constraints of working with several pathogens at the same time from a methodological standpoint and also to the tendency to simplify questions, a logical first approach to any scientifically oriented endeavor. Undoubtedly, the field of plant virology has been (and still is) particularly successful in providing novel and valuable knowledge that extends to the adoption of valid control measures against many viral diseases. Yet in most cases, there are still complexities that remain to be elucidated (Sanfaçon 2017), and we believe it is timely to recognize the importance of mixed viral infections. Here, we aim to summarize a selection of the most relevant literature available about the topic, with emphasis on the outcomes for each of the players involved, and we focus mainly on plant viruses transmitted by insects because they represent the major group of plant virus vectors. Nevertheless, we include a few examples of viruses disseminated through contact, or even several cases in which subviral agents participate in multiple infections, as mentioned above. This review is organized in three parts, referring to the impact of mixed infections with respect to viruses, vectors, and host plants. However, as a note of caution, this separation was adopted only to facilitate the classification of topics, and it is important to keep in mind that mixed infections often require the participation of the three mentioned players. Thus, the expected effects of mixed infections most likely will have consequences on each of the players, as represented schematically in Figure 1. The concluding remarks include a general discussion of the implications of mixed viral infections, addressing their importance for the ecology of viruses and for the control strategies against viral pathogens.

IMPACT ON VIRUSES

During a mixed viral infection, the viruses involved may interact between themselves in a range that goes from synergism to neutralism to antagonism, having a direct impact on the plant host and also potentially on their relationships with vector organisms. The outcome depends on the different aspects and parameters evaluated, such as the within-host interactions between viruses or the virus adaptation to the host (Mascia and Gallitelli 2016; Syller 2012). When there are no changes in viral accumulation (or their dynamics) during the mixed infection compared with the single infections with the individual viruses, the interaction is often

called neutral and the phenotypes (or symptoms in broad sense) can be the same as those observed in single infections, instead of the frequent occurrence of host responses with additive symptom expression (Mascia and Gallitelli 2016). On the other hand, if there are differences between the single and the mixed infections, the interactions can be classified as synergistic or antagonistic.

In a synergistic interaction, at least one of the viruses benefits from the presence of the other(s), which is manifested by an increase in the viral titer and/or pathogenicity (or other supra-level properties such as the capacity to disseminate efficiently by the vector organism) compared with a single infection. The resulting symptoms are often more severe than those observed in single

infections of the same viruses. There are several reasons that could explain, individually or combined, this effect (see Figs. 2 and 3 for schematic representations). This effect can be caused by an increase in replication, by the ability to invade new plant tissues, or even by interference with plant defenses (particularly, but not necessarily uniquely, with post-transcriptional gene silencing mechanisms). Consequently, synergisms may have a high economic impact when they occur in crops because the resulting diseases are likely to become more severe. As an example, we can cite the serious epidemic of cassava mosaic disease, which is caused by a mixed infection between isolates of *African cassava mosaic virus* and isolates of the Uganda strain of *East African*

FIGURE 1 Different scenarios of interaction during mixed viral infections of plants. Responses in terms of virus accumulation and temporal dynamics (left column), degree of symptoms in the susceptible host (middle column), and efficiency of vector transmission (right column) are schematically represented, respectively, for the individual infections by virus 1 or virus 2 in the first and second rows. The third row proposes one of the possible outcomes of a mixed infection of viruses 1 and 2, with increased and faster accumulation of one of the viruses (represented by number of virions and inclination of curves), causing more damage to the plant (less growth and severe symptoms) and altering their vector dissemination (efficiency proportional to vector size). For each of the parameters, combined outcomes might differ as shown in the lower part of the figure, where the type size of the numerals represents neutral, synergistic, antagonistic, or both types of responses for each virus. See the text for further details.

FIGURE 2

Schematic representation of interactions occurring at the cellular level during coinfection by two unrelated viruses (1 and 2) in a susceptible host plant. Cell entry and exit processes, either through the action of vector organisms (inoculation and acquisition) or through movement from and to adjacent cells (cell-to-cell transport through plasmodesmata, or invasion of distant tissues during long-distance transport), as well as essential intracellular viral processes (e.g., disassembly, gene expression, genome replication, and encapsidation of progeny) are represented by arrows of colors matching the individual viruses. The involvement of host factors and other processes is omitted for simplicity, as well as presumed differences in localization and timing of events. Outcomes of the positive and facilitative interactions are represented with arrows, whereas negative interactions are indicated by blockage symbols (T-shaped connectors); neutral effects are omitted for simplicity. Representative hypothetical examples of cross-assistance/complementation of movement functions, or transscapsidations during vector transmission, are represented by question marks. See the text for further details.

cassava mosaic virus, both members of the genus *Begomovirus* and leads to huge annual economic losses (Legg and Fauquet 2004). Many other cases are mentioned in Table 1. A frequent combination causing a synergistic interaction involves a potyvirus with different unrelated viruses, in most cases clearly favoring the second virus (Pruss et al. 1997), with the involvement of the potent RNA silencing suppressor helper-component proteinase (HC-Pro) of potyviruses (Valli et al. 2018). Interestingly, an exception is the SPVD mentioned in the introduction, in which the potyvirus partner SPFMV benefits from the presence of the crinivirus SPCSV (Karyeija et al. 2000); in this case, the RNA silencing function of SPFMV was shifted to a different gene product other than HC-Pro (Mingot et al. 2016; Untiveros et al. 2016).

There are some peculiarities in the interactions leading to conceptually clear synergisms that are not always easy to identify as such, because they affect traits beyond the measurable viral load. For instance, one kind of synergism could be the so-called helper dependence, in which one of the viruses involved is defective in one or more essential functions that in turn can be supplied by the other virus, the helper, acting as a provider or facilitator (Mascia and Gallitelli 2016). In an extreme example of this, one virus might become a sort of parasite of the other, such as in the interaction among *Groundnut rosette virus* (GRV), its satellite RNA (satRNA), and *Groundnut rosette assistor virus* (GRAV) that together cause groundnut rosette disease (GRD): GRV and its satRNA depend on the coat protein (CP) of GRAV to be encapsidated and transmitted by the aphid vector *Aphis craccivora* (Murant 1990). Another similar situation is the vector-mediated transmission by complementation in *trans*, also referred to as heterologous complementation or transcomplementation. In certain viruses, such as potyviruses and caulimoviruses, there are nonstructural proteins called helper components that are needed for vector transmission, acting as specific molecular bridges between the virus particles and the vector (Pirone and Blanc 1996). It has been observed in mixed infections involving potyviruses and other unrelated viruses that these latter ones may benefit from the action of helper components by being transmitted. For instance, *Potato aucuba mosaic virus* (PAMV) is the only known member of the genus *Potexvirus* that can be transmitted by aphids as a result of transcomplementation when present in a mixed-infected plant together with a potyvirus (Kassanis 1961; Manoussopoulos 2001).

On the other hand, in the frame of an antagonistic interaction the presence of more than one virus is detrimental for at least one of them, for instance with a measurable decrease in the viral load. In general, it has been postulated that most of the described antagonism is attributable to interactions, direct or indirect, between proteins from the viruses involved in the mixed infection (DaPalma et al. 2010; Díaz-Muñoz 2019). Compared with synergistic interactions, there are fewer examples of antagonism, which is expected because attenuation instead of exacerbation is more likely to remain unnoticed. Furthermore, in a theoretical situation of synergism plus antagonism (as in the last column of the lower panel in Fig. 1), it is more likely that the outcome was described as synergistic for the enhanced virus simply because not enough attention was paid to the alleviated partner. As well-documented examples, we can mention the suggested antagonism between isolates of *Potato virus X* (PVX) and of *Potato virus Y* (PVY) in tobacco (Ross 1950), as well as work in papaya with mixed infections between isolates of *Papaya ringspot virus* (PRSV) and of *Papaya mosaic virus* (PapMV) (Chávez-Calvillo et al. 2016). We can also mention the antagonism observed in tomato between isolates of *Tomato torrado virus* (ToTV) and of *Pepino mosaic virus* (PepMV), where the severity of ToTV-induced symptoms was unaffected but differences in virus accumulation were observed in mix-infected plants; the titer of ToTV slightly increased in the early phase of the infection, whereas that of PepMV was strongly reduced at all timepoints, including a pronounced decrease at later times. In this case, effects on the epidemiology of viruses have been reported (Gómez et al. 2009, 2010). As an additional case, antagonism was reported between two tobamoviruses, *Hibiscus latent Singapore virus* (HLSV) and *Tobacco mosaic virus* (TMV), in *Nicotiana benthamiana* plants; HLSV levels were decreased compared with a single infection, whereas TMV levels remained almost unaltered (Chen et al. 2012).

An interesting type of antagonistic interaction is cross-protection, also known as homologous interference or superinfection exclusion, which takes place when the presence of one virus prevents or interferes with a subsequent infection by another virus that is, generally, but not exclusively, homologous (Syller and Grupa 2016). In the context of agricultural activity, cross-protection can be very useful for biological control against viral diseases, mainly in cases where either no genetic resistance

FIGURE 3

Additional levels of interaction during viral mixed infections of plants. A schematic cross-section of a leaf (epidermis, parenchyma, and vasculature) is shown, together with the routes of hypothetical spread of infections by a virus capable of invading all tissues (virus 1, orange arrows) and another restricted to the vascular tissues (virus 2, blue arrows). Mixed infection might lead to interactions like those indicated by question marks: both facilitative and blocking outcomes of intracellular interactions (see Fig. 2) could influence dispersal of the viruses in the plant, and thus condition its effects (see Fig. 1). Furthermore, the behavior and performance of the vectors responsible for transmission (here represented as different insect vectors for each virus) might also respond positively or negatively to manipulative strategies (see Fig. 1). Arrows and T-shaped connectors represent positive and negative cross-interactions that can take place between viruses during mixed infections inside the plant and in the possible effect of one virus on the vector of the other virus. See the text for further details.

TABLE 1
Examples of mixed viral infections in plants caused by pairs of viruses

Virus 1 ^a	Virus 2 ^b	Host plants ^c	Comments	References
<i>Abutilon mosaic virus</i> (AbMV) (<i>Begomovirus</i>)	<i>Cucumber mosaic virus</i> (CMV) (<i>Cucumovirus</i>)	<i>Nicotiana benthamiana</i> and tomato (<i>Solanum lycopersicum</i>)	Synergism between RNA and single-strand DNA viruses; change in the tissue tropism of AbMV (no phloem limitation)	Wege and Siegmund (2007)
AbMV (<i>Begomovirus</i>)	<i>Tomato mosaic virus</i> (ToMV) (<i>Tobamovirus</i>)	<i>N. benthamiana</i> and tomato (<i>Solanum lycopersicum</i>)	Negative effect of ToMV on AbMV accumulation and a synergistic enhancement in pathogenicity	Pohl and Wege (2007)
<i>African cassava mosaic virus</i> (ACMV) (isolate ACMV-CM) (<i>Begomovirus</i>)	<i>East African cassava mosaic Cameroon virus</i> (EACMCV) (<i>Begomovirus</i>)	<i>N. benthamiana</i> and tobacco (<i>N. tabacum</i>) protoplasts	Synergism with an increase in symptom severity and viral accumulation of both viruses. Post-transcriptional gene silencing (PTGS) involved in the interaction	Vanitharani et al. (2004)
ACMV (<i>Begomovirus</i>)	Uganda strain of <i>East African cassava mosaic virus</i> (EACMV-UG) (<i>Begomovirus</i>)	Cassava (<i>Manihot esculenta</i>)	Synergism and cause of cassava mosaic disease. Field survey in Central African Republic	Zinga et al. (2013)
Bean pod mottle virus (BPMV) (<i>Comovirus</i>)	<i>Soybean mosaic virus</i> (SMV) (<i>Potyvirus</i>)	Soybean (<i>Glycine max</i>)	Synergism. Effects on biochemical traits of the plant and on behavior and performance of vectors	Lee and Ross (1972) and Peñaflor et al. (2016)
BPMV (<i>Comovirus</i>)	<i>Bean yellow mosaic virus</i> (BYMV) or <i>Peanut mottle virus</i> (PeMoV) (<i>Potyvirus</i>)	Soybean (<i>G. max</i>)	Neutralism	Anjos et al. (1992)
BYMV (<i>Potyvirus</i>)	A potyvirus strain of uncertain taxonomical status denominated <i>Pea mosaic virus</i> (<i>Potyvirus</i>)	Pea (<i>Pisum sativum</i>)	A nonaphid-transmissible isolate of BYMV that depends on mixed infection with the potyvirus strain pea mosaic potyvirus to be transmitted	Hobbs and McLaughlin (1990)
<i>Beet mild yellowing virus</i> (BMV) (<i>Polerovirus</i>)	<i>Groundnut rosette virus</i> (GRV) (<i>Umbravirus</i>)	<i>N. clevelandii</i> , <i>N. benthamiana</i>	BMV became mechanically transmissible	Mayo et al. (2000)
<i>Cassava brown streak virus</i> (CBSV) (<i>Ipomovirus</i>)	<i>Ugandan cassava brown streak virus</i> (UCBSV) (<i>Ipomovirus</i>)	Cassava (<i>M. esculenta</i>)	Synergism and cause of cassava brown streak disease	Jacobson et al. (2018) and Rey and Vanderschuren (2017)
<i>Cowpea chlorotic mottle virus</i> (CCMV) (<i>Bromovirus</i>)	SMV, PeMoV (<i>Potyvirus</i>), or <i>Tobacco ringspot virus</i> (TRSV) (<i>Nepovirus</i>)	Soybean (<i>G. max</i>)	Effect of mixed infections on plant characteristics and chemical composition. No synergism observed	Demski and Jellu (1975)
CMV (<i>Cucumovirus</i>)	<i>Blackeye cowpea mosaic potyvirus</i> (BICMV) (<i>Potyvirus</i>)	Cowpea (<i>Vigna unguiculata</i>), <i>Cassia obtusifolia</i> , cucumber (<i>Cucumis sativus</i>), zucchini (<i>Cucurbita pepo</i>), <i>G. max</i> , <i>N. tabacum</i> , <i>Phaseolus lunatus</i> , bean (<i>Phaseolus vulgaris</i>)	Synergism and cause of cowpea stunt disease. Can reduce yield of cowpea in the greenhouse. Aphids can transmit both viruses from mixed-infected plants	Pio-Ribeiro et al. (1978)
CMV (<i>Cucumovirus</i>)	BICMV (<i>Potyvirus</i>)	Cowpea (<i>V. unguiculata</i>)	Synergism. Severe symptoms might not be only attributable to an increase in CMV levels	Anderson et al. (1996)

(Continued on next page)

^a Viruses are listed in alphabetical order with their acronym and include the taxonomic adscription (genus) in parentheses.

^b When the bibliographical references include data for more than a pair of viruses, the second partner of every combination is mentioned but is separated with a comma.

^c Common names and scientific names are used to identify hosts.

mechanisms are known or they cannot be easily implemented. In such scenarios, avirulent or mild protecting viruses can be inoculated to crop plants to prevent aggressive viral infections in the field, acting as a kind of “vaccine” and reducing economic losses. Successful cases of cross-protection are the control of

Citrus tristeza virus, *Zucchini yellow mosaic virus* (ZYMV), and PRSV (Ziebell and Carr 2010).

An important factor that influences the outcome of a mixed infection is the moment at which the viruses arrive and infect the host. Coinfection is defined when the viruses simultaneously

TABLE 1
(Continued from previous page)

Virus 1 ^a	Virus 2 ^b	Host plants ^c	Comments	References
CMV (<i>Cucumovirus</i>)	<i>Turnip mosaic virus</i> (TuMV) (Potyvirus)	<i>N. benthamiana</i>	Synergism. Analysis of the CMV 2b protein function in the mixed infection	Takeshita et al. (2012)
CMV (<i>Cucumovirus</i>)	<i>Pepper mottle virus</i> (PepMoV) (Potyvirus)	Pepper (<i>Capsicum annuum</i>)	Synergism. Detailed evaluation of symptom severity and statistical proof of synergy	Murphy and Bowen (2006)
CMV (Fny-CMV and LS-CMV) (<i>Cucumovirus</i>)	<i>Watermelon mosaic virus</i> (WMV) (Potyvirus)	Zucchini (<i>Cucurbita pepo</i>)	Synergism with a high increase of CMV levels	Wang et al. (2002)
CMV (Fny-CMV and LS-CMV) (<i>Cucumovirus</i>)	<i>Zucchini yellow mosaic virus</i> (ZYMV) (Potyvirus)	Zucchini (<i>Cucurbita pepo</i>), melon (<i>Cucumis melo</i>)	Strong synergistic pathological responses	Wang et al. (2002)
CMV (Fny-CMV) (<i>Cucumovirus</i>)	ZYMV (Potyvirus)	Cucumber (<i>Cucumis sativus</i>), bottle gourd (<i>Lagenaria siceraria</i>)	Quantification of synergism by real-time reverse transcription (RT)-PCR	Zeng et al. (2007)
CMV (impaired in long-distance, strain M-CMV) (<i>Cucumovirus</i>)	ZYMV (strains ZYMV-A and ZYMV-AG) (Potyvirus)	Zucchini (<i>Cucurbita pepo</i>)	Synergism in pathology and gain of long-distance movement for M-CMV	Choi et al. (2002)
<i>Lettuce infectious yellows virus</i> (LIYV) (<i>Crinivirus</i>)	TuMV (Potyvirus)	<i>N. benthamiana</i>	Synergistic interaction with enhanced accumulation of LIYV, mediated by the entire TuMV or only the TuMV P1/helper-component proteinase (HC-Pro) sequence	Wang et al. (2009a, b)
<i>Maize chlorotic mottle virus</i> (MCMV) (<i>Machlomovirus</i>)	<i>Maize dwarf mosaic virus A</i> (MDMV-A) (Potyvirus)	Maize (<i>Zea mays</i>), sorghum (<i>Sorghum vulgare</i>), wheat (<i>Triticum aestivum</i>)	Synergism and cause of corn lethal necrosis disease (CLND). Field survey in Kansas, U.S.A.	Uyemoto et al. (1980)
MCMV (<i>Machlomovirus</i>)	<i>Wheat streak mosaic virus</i> (WSMV) (Tritimovirus)	Maize (<i>Z. mays</i>), sorghum (<i>Sorghum vulgare</i>), wheat (<i>T. aestivum</i>)	Synergism and cause of CLND. Field survey in Kansas	Uyemoto et al. (1980)
MCMV (<i>Machlomovirus</i>)	<i>Sugarcane mosaic virus</i> MD-B (SCMV-MD-B) (formerly known as <i>Maize dwarf mosaic virus</i> [MDMV-B]) (Potyvirus)	Maize (<i>Z. mays</i>)	Synergism with high increase of MCMV levels. Decrease in chlorophyll levels and in the ratio of chloroplast to cytoplasmic rRNA	Goldberg and Brakke (1987)
MCMV (<i>Machlomovirus</i>)	WSMV (Tritimovirus)	Maize (<i>Z. mays</i>)	Synergism and cause of corn lethal necrosis (now called maize lethal necrosis [MLN]). WSMV infections were enhanced by MCMV and more pronounced at higher temperatures	Scheets (1998)
MCMV (<i>Machlomovirus</i>)	SCMV, MDMV, or <i>Johnsongrass mosaic virus</i> (JGMV) (all Potyvirus)	Maize (<i>Z. mays</i>)	Synergism caused by coinfection of MCMV with any one of the indicated Potyvirus viruses. The resulting disease is known as MLN and causes high economic losses	Stewart et al. (2017)

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infect the host, arriving at roughly the same time, whereas superinfection refers to mixed infections where the viruses arrive at different moments (Saldaña et al. 2003). In nature, the occurrence of each one depends on the viral density and the number of

healthy hosts available during the epidemic episode: at the beginning, with low viral densities and a high number of uninfected plants, the occurrence of coinfections can be favored; later, as the diseases spread and the number of healthy host plants

TABLE 1
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Virus 1 ^a	Virus 2 ^b	Host plants ^c	Comments	References
<i>Papaya ringspot virus</i> (PRSV) (Potyvirus)	<i>Papaya mosaic virus</i> (PapMV) (Potexvirus)	Papaya (<i>Carica papaya</i>)	Superinfection (first PapMV) causes antagonism, but coinfection or superinfection (first PRSV) causes synergism	Chávez-Calvillo et al. (2016)
<i>Parsnip yellow fleck virus</i> (PYFV) (Sequivirus)	<i>Anthriscus yellows virus</i> (AYV) (Waikavirus)	Parsnip (<i>Pastinaca sativa</i>)	PYFV depends on mixed infection with AYV to be aphid transmitted. Electron microscopy to detect viral particles in aphids	Murant and Goold (1968) and Murant et al. (1976)
PeMoV (Potyvirus)	TRSV (Nepovirus)	Soybean (<i>G. max</i>)	Effect of mixed infection on plant characteristics and chemical composition. No synergism observed	Demski and Jellu (1975)
<i>Pepper huasteco yellow vein virus</i> (PHYVV) (Begomovirus)	<i>Pepper golden mosaic virus</i> (PepGMV) (Begomovirus)	Pepper (<i>Capsicum annuum</i>)	Synergism. Analysis of symptom severity, viral DNA concentration, and tissue localization	Rentería-Canett et al. (2011)
<i>Plum pox virus</i> (PPV) (Potyvirus)	<i>Tobacco etch virus</i> (TEV) (Potyvirus), <i>Potato virus X</i> (PVX) (Potexvirus), or CMV (Cucumovirus)	Tobacco (<i>N. tabacum</i>), <i>N. benthamiana</i> , <i>N. clevelandii</i>	Function of HC-Pro in the long-distance movement and in the control of the host range of potyviruses	Sáenz et al. (2002)
<i>Potato leaf roll virus</i> (PLRV) (Polerovirus)	<i>Tobacco mosaic virus</i> (TMV) (Tobamovirus), <i>Potato spindle tuber viroid</i> (PSTVd) (Pospiviroid), <i>Alfalfa mosaic virus</i> (AMV) (Alfamovirus), <i>Potato virus M</i> (PVM) (Carlavirus), <i>Potato virus S</i> (PVS) (Carlavirus), <i>Potato virus T</i> (PVT) (Tepovirus), or <i>Potato virus X</i> (PVX) (Potexvirus)	<i>Solanum brevidens</i>	<i>Solanum brevidens</i> is tolerant to PLRV and accumulation of this virus did not vary in mixed infections with any of the viruses or viroids tested	Valkonen (1992)
PLRV (Polerovirus)	<i>Pea enation mosaic virus-2</i> (PEMV-2) (Umbravirus), CMV (Cucumovirus), PVX (Potexvirus), or GRV (Umbravirus)	<i>N. benthamiana</i> , <i>N. clevelandii</i>	PLRV gains mechanical transmissibility when in mixed infections with PEMV-2 or GRV	Mayo et al. (2000)
PLRV (Polerovirus)	PEMV-2 (Umbravirus), CMV (Cucumovirus), PVX (Potexvirus), TMV (Tobamovirus), or <i>Potato virus Y</i> (PVY) (Potyvirus)	<i>N. benthamiana</i> , <i>N. clevelandii</i>	PEMV-2 complements PLRV mechanical transmission and facilitates its full systemic infection (cell-to-cell and inside-phloem movement)	Ryabov et al. (2001)
PVX (Potexvirus)	PVY (Potyvirus)	Tobacco (<i>N. tabacum</i>)	Synergistic interaction with an increase of infectious PVX particles, probably related to an alteration in the relative levels of (–) and (+) strands of PVX RNA	Vance (1991)

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decrease gradually, while the viral density increases, superinfections become more frequent (Miralles et al. 2001; Saldaña et al. 2003). The moment of infection might also influence virus evolution; for instance, virus fitness might increase in coinfections

and not in superinfections (Miralles et al. 2001). In addition, the outcome of superinfections may be dramatically influenced by aspects such as the order of infection of the viruses involved and the timeframe between the infections; for both coinfections

TABLE 1
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Virus 1 ^a	Virus 2 ^b	Host plants ^c	Comments	References
PVX (<i>Potexvirus</i>)	<i>Tobacco vein mottling virus</i> (TVMV), TEV, PepMoV, or PVY (<i>Potyvirus</i>)	Tobacco (<i>N. tabacum</i>)	Synergism. Alteration in the replication of PVX RNA attributable to the expression of 5'-proximal sequences of either TVMV or TEV	Vance et al. (1995)
PVX (<i>Potexvirus</i>)	PVS (<i>Carlavirus</i>)	Potato (<i>Solanum tuberosum</i>)	Increase of PVS titer and disease symptoms. More than 30 potato cultivars tested	Nyalugwe et al. (2012)
PVX (<i>Potexvirus</i>)	PVY (N-Wi strain) (<i>Potyvirus</i>)	<i>N. benthamiana</i> , tobacco (<i>N. tabacum</i>)	Synergism. Quantification of symptom expression and levels of coat proteins and suppressors of silencing under different temperature conditions and during co- or superinfection	Senanayake and Mandal (2014)
PVX (<i>Potexvirus</i>)	Potato virus A (PVA) (<i>Potyvirus</i>)	<i>N. benthamiana</i>	HC-Pro from PVA mediates reductions on methionine and glutathione and has a crucial role in the synergism observed	De et al. (2018)
PVY (<i>Potyvirus</i>)	CMV (<i>Cucumovirus</i>)	Tomato (<i>Solanum lycopersicum</i>)	Increase of both PVY and CMV levels. Differences with the same mixed infection in tobacco and cucumber	Mascia et al. (2010)
PVY (<i>Potyvirus</i>)	TEV (<i>Potyvirus</i>)	Pepper (<i>Capsicum annuum</i>)	A nonaphid-transmissible strain of TEV depends on mixed infection with PVY to be aphid transmitted	Simons (1976)
PVY (<i>Potyvirus</i>)	PSTVd (Pospiviroid)	Potato (<i>Solanum tuberosum</i>)	Synergism only in coinfection or superinfection with PSTVd infecting prior to PVY. PVY levels significantly higher in mixed-infected plants	Singh and Somerville (1987)
PVY (<i>Potyvirus</i>)	TMV (<i>Tobamovirus</i>), PSTVd (<i>Pospiviroid</i>), AMV (<i>Alfamovirus</i>), PVM (<i>Carlavirus</i>), PVS (<i>Carlavirus</i>), PVT (<i>Tepovirus</i>), or PVX (<i>Potexvirus</i>)	<i>Solanum brevidens</i>	Accumulation of PVY in mixed-infected plants with TMV and PSTVd increased up to 1,000-fold, whereas no changes were observed in coinfections with the other viruses tested. <i>Solanum brevidens</i> is tolerant to PVY and PVX, which explains differences with other cases between PVX and PVY (usually synergistic)	Valkonen (1992)
PVY (<i>Potyvirus</i>)	PLRV (<i>Polerovirus</i>)	Potato (<i>Solanum tuberosum</i>)	Synergism with more severe symptoms. Effect of the mixed infection on the fecundity and preference of two aphid vectors of PVY and PLRV	Srinivasan and Alvarez (2007)

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TABLE 1
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Virus 1 ^a	Virus 2 ^b	Host plants ^c	Comments	References
<i>Southern cowpea mosaic virus</i> (SCPMV) (<i>Sobemovirus</i>)	<i>Southern bean mosaic virus</i> (SBMV) (<i>Sobemovirus</i>)	Cowpea (<i>V. unguiculata</i>), bean (<i>Phaseolus vulgaris</i>)	In coinfection, the host range of SCPMV is modified but the range of SBMV is not	Hacker and Fowler (2000)
<i>Southern rice black-streaked dwarf virus</i> (SRBSDV) (<i>Fijivirus</i>)	<i>Rice ragged stunt virus</i> (RRSV) (<i>Oryzavirus</i>)	Rice (<i>Oryza sativa</i>)	Synergism. Increase in replication and movement of viruses and inhibition of host immunity. Alteration on vector selection preferences tending to enhance mixed infection	Li et al. (2017) and Wang et al. (2014)
SMV (<i>Potyvirus</i>)	TRSV (<i>Nepovirus</i>) or PeMoV (<i>Potyvirus</i>)	Soybean (<i>G. max</i>)	Effect of mixed infections on plant characteristics and chemical composition. No synergism observed	Demski and Jellu (1975)
SMV (<i>Potyvirus</i>)	BPMV (<i>Comovirus</i>)	Soybean (<i>G. max</i>)	Synergism with increase of BPMV. Localization by electron microscopy. Reduction in yields both in the field and greenhouse	Anjos et al. (1992) and Calvert and Ghabrial (1983)
SMV (<i>Potyvirus</i>)	<i>Cowpea mosaic virus</i> (CPMV) (<i>Comovirus</i>)	Soybean (<i>G. max</i>)	Synergism with increase of CPMV. Localization by electron microscopy	Anjos et al. (1992)
<i>Strawberry pallidosis-associated virus</i> (SPaV) (<i>Crinivirus</i>)	<i>Beet pseudo-yellows virus</i> (BPYV) (<i>Crinivirus</i>)	Strawberry (<i>Fragaria × ananassa</i>)	Causal agents of strawberry pallidosis disease. Detection in field surveys in North America and Peru	Tzanetakis et al. (2006) and Wintermantel et al. (2006)
<i>Sweet potato chlorotic stunt virus</i> (SPCSV) (<i>Crinivirus</i>)	<i>Sweet potato feathery mottle virus</i> (SPFMV) (<i>Potyvirus</i>)	Sweet potato (<i>Ipomoea batatas</i>)	Together they cause the sweet potato virus disease, a synergistic interaction with a 600-fold increase of SPFMV and severe symptoms	Karyeija et al. (2000)
SPCSV (<i>Crinivirus</i>)	<i>Sweet potato mild mottle virus</i> (SPMMV) (<i>Ipomovirus</i>)	Sweet potato (<i>I. batatas</i>)	Synergism. Causal agents of sweet potato severe mosaic disease	Mukasa et al. (2003) and Tugume et al. (2005)
TEV	TMV (<i>Tobamovirus</i>), CMV (<i>Cucumovirus</i>), or PVX (<i>Potexvirus</i>)	<i>N. benthamiana</i> , tobacco (<i>N. tabacum</i>)	Synergism. TEV P1/HC-Pro protein enhances the pathogenicity and accumulation of both TMV and CMV. Transactivation of PVX replication	Pruss et al. (1997)
TMV (<i>Tobamovirus</i>)	<i>Hibiscus latent Singapore virus</i> (HLSV) (<i>Tobamovirus</i>)	<i>N. benthamiana</i>	Evolutionary game theory to model the coinfection between TMV and HLSV and validation by quantitative RT-PCR	Chen et al. (2012)
<i>Tomato chlorosis virus</i> (ToCV) (<i>Crinivirus</i>)	<i>Tomato spotted wilt virus</i> (TSWV) (<i>Tospovirus</i>)	Tomato (<i>Solanum lycopersicum</i>)	Synergism increases ToCV while TSWV does not change. Breakdown of resistance against TSWV in plants preinfected with ToCV, contrarily to coinfection	García-Cano et al. (2006)
ToCV (<i>Crinivirus</i>)	<i>Tomato infectious chlorosis virus</i> (TICV) (<i>Crinivirus</i>)	<i>Physalis wrightii</i> , <i>N. benthamiana</i>	Effects of coinfection in virus accumulation and transmission efficiency are host specific	Wintermantel et al. (2008)

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and superinfections, differences in the capacity of viruses to invade or not invade certain tissues have been observed. Next, a series of examples are provided to illustrate the different outcomes that can result.

In the previously mentioned work in papaya, the outcome of the mixed infection was different when the plant was infected first with PapMV and then with PRSV, causing the antagonism mentioned; if either the order was reversed or the viruses arrived simultaneously, the result was synergism (Chávez-Calvillo et al. 2016). Similarly, the example given above of an antagonistic interaction between HLSV and TMV occurred in the frame of a coinfection, but the authors mentioned that the presence of HLSV in a superinfection protected the host against a late arrival of TMV, thus acting in practical terms as a cross-protection event (Chen et al. 2012). Another example of conditional outcome derives from one of the first synergisms described: tobacco plants infected with isolates of PVY and PVX (Damirdagh and Ross 1967; Goodman and Ross 1974; Smith 1931; Vance 1991). When PVX was inoculated on tobacco previously infected with PVY, the levels of accumulation of PVX increased considerably compared with the single-infected control. However, this synergism was compromised if the time between the infections with the two viruses was >24 h; it was later demonstrated that the silencing suppressor of the potyvirus PVY (HC-Pro) was one of the factors involved in the outcome of the synergism (González-Jara et al. 2005). As a final example of the different relationships occurring between viruses

in mixed infections, we can cite work in tomato plants involving mixed infections of *Tomato chlorosis virus* (ToCV) and *Tomato spotted wilt virus* (TSWV) that resulted in a synergism that can even end with the death of susceptible plants. In those plants, there was a high increase in ToCV accumulation, whereas TSWV levels remained unaltered. Coinfection of TSWV-resistant plants with ToCV and TSWV did not alter the resistance behavior of the plants; however, and most interestingly, when the resistant plants were challenged in a superinfection with ToCV arriving before TSWV, the resistance was lost. This observation suggested that ToCV might need a certain amount of time to affect the defense response in the resistant plants, such as requiring enough time to express some proteins acting as suppressors of RNA silencing, or to reach a certain accumulation level (García-Cano et al. 2006). However, interference with the plant defense mechanisms by viral proteins is likely not the only component that determines whether an interaction will be synergic or antagonistic: some proteins may facilitate the replication, intercellular movement, and/or the within-host spread of the other virus (Elena et al. 2014).

Mixed infections by the same combination of viruses but in different host plants may also lead to different outcomes in virus accumulation levels, host response, and transmission by vector organisms, for example. We will consider these aspects in different sections, but it is relevant to stress that virus–virus interactions are highly dependent on the biological environment (i.e., the

TABLE 1
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Virus 1 ^a	Virus 2 ^b	Host plants ^c	Comments	References
ToCV (<i>Crinivirus</i>)	<i>Tomato severe rugose virus</i> (ToSRV) (<i>Begomovirus</i>)	Tomato (<i>Solanum lycopersicum</i>)	Near 50% of mixed infections in field surveys, with symptoms of ToSRV enhanced in the top part of plants, and crinivirus-like symptoms at the bottom	Macedo et al. (2014)
<i>Tomato leaf curl New Delhi virus</i> (ToLCNDV) (<i>Begomovirus</i>)	<i>Tomato leaf curl Palampur virus</i> (ToLCPaV) (<i>Begomovirus</i>)	Tomato (<i>Solanum lycopersicum</i>), cucumber (<i>Cucumis sativus</i>)	Genetic reassortment and heteroencapsidation. Whitefly transmission of pseudorecombinants	Kanakala et al. (2013)
<i>Tomato rugose mosaic virus</i> (ToRMV) (<i>Begomovirus</i>)	<i>Tomato yellow spot virus</i> (ToYSV) (<i>Begomovirus</i>)	<i>N. benthamiana</i> , tomato (<i>Solanum lycopersicum</i>)	ToYSV induces a change in tissue tropism of ToRMV (exits phloem). Both positive (synergism) and negative (interference) interactions are observed depending on the infection stage	Alves-Júnior et al. (2009)
TuMV (<i>Potyvirus</i>)	<i>Cauliflower mosaic virus</i> (CaMV) (<i>Caulimovirus</i>)	<i>Arabidopsis thaliana</i>	Effect of mixed infection on virus infectivity, pathogenicity, and accumulation. No symptom synergism	Martín and Elena (2009)
WSMV (<i>Tritimovirus</i>)	<i>Triticum mosaic virus</i> (TriMV) (<i>Poacevirus</i>)	Wheat (<i>T. aestivum</i>)	Cultivar-specific synergism and influence of different temperatures. Determination of different physiological parameters. Impact on endogenous and viral small RNA profiles	Byamukama et al. (2012) and Tatineni et al. (2010, 2014)
ZYMV (<i>Potyvirus</i>)	CMV (<i>Cucumovirus</i>)	Zucchini (<i>Cucurbita pepo</i>)	Synergism with increase of CMV levels	Fattouh (2003)
ZYMV (nonaphid-transmissible isolate ZYMV-NAT) (<i>Potyvirus</i>)	PRSV (<i>Potyvirus</i>)	Melon (<i>Cucumis melon</i>)	Heteroencapsidation and gain of aphid transmissibility	Bourdin and Lecoq (1991)

common host) where they are present together. As an example, we can cite a mixed infection between two criniviruses, ToCV and *Tomato infectious chlorosis virus* (TICV), in two different host plants: *Physalis wrightii* and *N. benthamiana* (Wintermantel et al. 2008). Compared with single infections, the titer of both viruses decreased in mixed infections of *P. wrightii*, whereas TICV titers increased and ToCV decreased in *N. benthamiana*. Vector transmission efficiencies by *Trialeurodes vaporariorum* were directly correlated with the accumulation levels of both viruses, with more transmission of TICV from mixed-infected plants (this case fits in the proposed effect of the last column in Fig. 1). Even more interestingly, vector *T. abutilonea* was able to transmit TICV from mixed-infected plants, even if it was described as a nonvector for this virus alone (Duffus et al. 1996). This suggests that a complementation for transmission might have taken place, with ToCV providing one or more factors necessary for the *T. abutilonea*–TICV association and subsequent transmission in a process independent of host factors, as the virus was transmitted from both hosts (Wintermantel et al. 2008).

The interaction between viruses inside a host cell (as represented schematically in Fig. 2) can be highly influenced by the multiplicity of infection (MOI), which indicates the number of viral genomes present in the same cell (Gutiérrez et al. 2010). There are some indications that the MOI is controlled by virus-encoded functions, such as the suppression of RNA silencing mechanisms (Donaire et al. 2016). In mixed infections of *N. benthamiana* with two genotypes of TMV, the MOI was higher at initial stages and then changed (González-Jara et al. 2009). Determining the values of the MOI and how they are regulated can help to decipher the relationships between dynamics of virus populations and the control of genome copy numbers (Gutiérrez et al. 2012). Beyond the cellular level but closely related to this topic is the tissue tropism or the specificity of each virus to infect only some cell types or tissues (Mascia and Gallitelli 2016). During infection of a host plant, viruses might be found in different cells, from all types to only those found in vascular tissues, or exhibit restrictions to enter meristems or invade seeds. In the case of mixed viral infections, we could expect modifications and changes in host responses that might condition the pathology of the viruses involved. These responses, for instance, might alter the tissue tropism, a phenomenon observed mainly in phloem restricted viruses (Mascia and Gallitelli 2016). In single infections of *N. benthamiana* plants, *Tomato yellow spot virus* (ToYSV) localizes in mesophyll cells, whereas *Tomato rugose mosaic virus* (ToRMV) is restricted to the phloem. However, when the plant is infected by both ToYSV and ToRMV, the localization of ToRMV changes and the virus becomes able to invade mesophyll cells as well (Alves-Júnior et al. 2009). Also in *N. benthamiana*, mixed infections between isolates of the potyvirus *Potato virus A* (PVA) and of the luteovirus *Potato leaf roll virus* (PLRV) allow the latter virus to infect different cell types in leaves, whereas it is restricted to the phloem in single infection. A possible explanation would be that movement proteins from PVA could complement movement deficiencies of PLRV, in a mechanism independent of the RNA silencing suppression function of HC-Pro (Savenkov and Valkonen 2001). Similarly, in *N. clevelandii* and *N. benthamiana* plants infected with both PLRV and the umbravirus *Pea enation mosaic virus-2* (PEMV-2), PLRV gained the capacity to be mechanically transmitted because it could be located in mesophyll cells. Again, a modification in the cell-to-cell movement and the interference with the host RNA silencing mechanisms by PEMV-2 may explain this observation (Ryabov et al. 2001). Additional examples of similar outcomes are mixed infection by the begomovirus *Abutilon mosaic virus* (AbMV) and either the potyvirus *Cowpea aphid-borne mosaic virus* or the tombusvirus *Artichoke mottled crinkle virus*, which occasionally provoked the localization of AbMV in tissues other than the phloem

(Sardo et al. 2011), and the case of a movement-deficient strain of *Cucumber mosaic virus* (CMV) in zucchini squash that assisted in systemic spread by coinfection with a strain of the potyvirus ZYMV (Choi et al. 2002).

Besides these modifications in tissue tropism and virus distribution within the host, in some cases the mixed viral infection can even alter the host range. An example is the case of two sobemoviruses, *Southern cowpea mosaic virus* (SCPMV) and *Southern bean mosaic virus* (SBMV). SCPMV can infect cowpea (*Vigna unguiculata*) but not common bean (*Phaseolus vulgaris*), whereas SBMV can infect common bean but not cowpea. Even if the genomic RNA of SCPMV can replicate in bean cells, it cannot assemble viral particles. However, in coinfection with SBMV, it was observed that SCPMV accumulated in an encapsidated form in both inoculated and systemic bean leaves, suggesting a phenomenon of heterologous encapsidation or genome masking. Nevertheless, when the reciprocal experiment was done, the authors observed that SCPMV did not complement the host range restriction of SBMV in cowpea. Thus, in practice, the presence of SBMV modified the host range of SCPMV, allowing it to systemically infect bean, but SCPMV did not complement the host range restriction of SBMV, as it was unable to systemically infect cowpea (Hacker and Fowler 2000).

IMPACT ON VECTORS

Vectors are key players in many virus infection cycles, as a majority of plant viruses rely on them for dispersion. The virus transmission process by a vector organism involves finding the infected plant, acquisition (often through probing or feeding, as in the case of some phytophagous vectors), transport, and finally inoculation or delivery of virus to a new host plant. Vectors are the mobile partners in a plant–virus–plant interaction leading to dispersal of infection. Thus, the preferences of the vectors for one plant or another can condition the virus spread process. The importance of mixed viral infections in aphid transmission of plant viruses was reported previously in a review article rich in examples (Rochow 1972), addressing points such as the behavior of vectors or even recombination and transcapsidation events (these concepts are discussed in more detail in the previous section). All of these key concepts might have an impact on virus evolution.

Some works have also shown that vector behavior and/or physiology can be influenced by changes in the plant induced by viruses, which can in turn condition the spread and epidemiology of the viruses (Bak et al. 2017; Bosque-Pérez and Eigenbrode 2011). Therefore, in the case of mixed infections, the possibility of manipulative strategies must be considered. For instance, a surprising example of vector manipulation by viruses was described in rice plants and reflects the relationships of two reoviruses, *Southern rice black-streaked dwarf virus* (SRBSDV) and *Rice ragged stunt virus* (RRSV), with their insect vectors: the white-backed planthopper (WBPH; *Sogatella furcifera*) transmits SRBSDV, whereas the brown planthopper (BPH; *Nilaparvata lugens*) transmits RRSV (Wang et al. 2014). Preference assays using a Y-shape olfactometer-based device demonstrated that plants infected with SRBSDV modified the behavior not only of its vector WBPH but also of the nonvector BPH. Virus-free WBPHs were more attracted to infected plants than viruliferous insects, and viruliferous insects were more attracted to healthy plants than to infected ones: this result is consistent with manipulation of the vector to increase virus spread through virus-induced host modifications. Interestingly, the preference assays with BPHs, a nonvector of SRBSDV, showed only preference of rice plants infected with SRBSDV over a healthy plant, when they were viruliferous for RRSV, therefore favoring the occurrence of mixed infections. Further experiments are needed to elucidate the mechanisms involved in this behavior and to explain why viruses may manipulate host selection in order to favor the existence of mixed infections.

The apparent manipulation of the vector by the virus often triggers an increase in transmission rates and consequently in virus spread, which ensures the existence of the virus species in the long term. In other words, virus acquisition will be increased by vector attraction to infected plants, and transmission will be improved by attraction to healthy plants where the virus can be inoculated. This concept is known as the vector manipulation hypothesis, and it was proposed to explain the different approaches employed by the pathogen to enhance its dissemination (Ingwell et al. 2012). The virus may directly influence the vector and modify its behavior, mainly in viruses that are transmitted in a persistent way while the virus is inside the body of the vector, as in the case of the thrip *Frankliniella occidentalis* (Pergande) whose feeding behavior is altered when infected by TSWV: infected males feed more than uninfected ones, thus increasing the possibility of virus inoculation (Stafford et al. 2011). This proposed general concept is still awaiting further studies to verify how common it is, and it will be especially interesting to measure transmission efficiencies and vector behavior parameters in mixed infections. For instance, a recent research effort in our laboratory incorporated measurements of aphid feeding behavior through electrical penetration graphs, finding that a particular subphase related to virus acquisition was prolonged by aphids feeding on melon plants coinfecting by the potyvirus *Watermelon mosaic virus* (WMV) and the crinivirus *Cucurbit yellow stunting disorder virus* (CYSDV) compared with single-infected controls. Interestingly, this behavior might compensate a reduction in WMV load, thus ensuring efficient transmission of the potyvirus (Domingo-Calap et al. 2019).

Viruses may also indirectly influence vectors as a result of modifications in the host infected plant that will have an impact on the vector (Dietzgen et al. 2016). Manipulations exerted by viruses often result in a positive or neutral effect on transmission by vectors, and they are similar between different viruses depending on their mode of transmission (described in more detail in the next section) (Mauck et al. 2012). For example, we can cite the mixed infection of PVY and PLRV in potato plants that altered the performance of their most effective vectors, the aphids *Myzus persicae* and *Macrosiphum euphorbiae*. For both vectors, fecundity increased in mixed-infected potato plants, as did their preference for those plants (Srinivasan and Alvarez 2007). According to Srinivasan and Alvarez (2007), this behavior could be explained, at least in part, by an increase in sugars and amino acids in the phloem as a result of an inhibited phloem transport in mixed-infected compared with single-infected plants. In addition, it is likely that both viruses, when together, somehow manipulate the plant to make it more attractive to insects by visual and olfactory clues. Because mixed infections of PVY and PLRV are quite frequent in potato plants, the quality of hosts and the behavior of vectors during this interaction could have important epidemiological repercussions (Chatzivassiliou et al. 2008). Another example of this kind of multiple-level interaction involving vectors is provided by the above-mentioned mixed infection of WMV and CYSDV in melon. Our work showed a late time-recovery phenotype of the mixed-infected plants after a first onset of strong synergistic damage, resulting in a better condition and development and thus in more foliage surface available for acquisition of the viruses by vectors (Domingo-Calap et al. 2019).

An interesting feature related to mixed infections is that some viruses gain the ability to be transmitted by vectors that are not their natural vectors when they are infecting a susceptible plant alone. As we mentioned previously, the case of PAMV showed that a virus belonging to a taxonomic group (potexviruses) characterized by only contact dissemination can acquire an efficient vector-mediated transmissibility during mixed infection with a potyvirus. The key molecular feature here was the presence of a conserved DAG motif, typical of potyviruses, in the potexvirus, as demon-

strated in an elegant work through mutagenesis of the PVX CP to engineer the same aphid-transmissibility feature (Baulcombe et al. 1993).

Acquisition of new vector capacities was seen also in mixed infections by viruses from the family *Luteoviridae*, or in coinfections of unrelated viruses with a luteovirus. The general phenomenon can be named genomic masking or transcapsidation, and basically consists of a viral genome being encapsidated by the capsids of another virus present at the same time in the plant, generating a sort of chimeric new entity with shared properties (represented in Fig. 2 by orange and blue or blue and orange virus particles). Examples of this were observed in mixed-infected plants in which a virus gained transmissibility by different species of vectors. For instance, the aphid *Sitobion avenae* is a good vector of the MAV strain of the luteovirus *Barley yellow dwarf virus* (BYDV-MAV) in single infections, whereas the aphid *Rhopalosiphon padi* specifically transmits the RPV strain of the polerovirus *Cereal yellow dwarf virus* (CYDV-RPV). However, in plants infected by both viruses, BYDV-MAV also became transmissible by *R. padi* because the RNA of BYDV-MAV was encapsidated inside CYDV-RPV capsids, which conferred the transmissibility by *R. padi* (Creamer and Falk 1990; Rochow 1970). Similar results were described in oat and wheat infected by two strains of BYDV (BYDV-MAV and BYDV-PAV), where alterations in transmission specificity and synergism in symptoms were observed in addition to transcapsidation (Baltenberger et al. 1987; Creamer and Falk 1990).

The transcapsidation phenomenon is not restricted to luteoviruses and was also observed in viruses of the genus *Umbravirus* and some luteovirus-associated RNAs (specific subviral RNA replicons) that do not encode a CP. Their aphid-transmission capacity is absolutely dependent on transcapsidation with other coinfecting luteovirus (Casteel and Falk 2016; Syller 2000).

It is worth mentioning here a related situation known as heteroencapsidation, where a virus genome can partially acquire the properties of the particles of another virus through heterologous encapsidation. For instance, electron microscopy was used to demonstrate that an aphid nontransmissible isolate of the potyvirus ZYMV can produce heteroencapsidated particles in mixed infection with the potyvirus PRSV and thus be efficiently transmitted by aphids (Bourdin and Lecoq 1991). Other well-documented examples involve whitefly-transmitted begomovirus from the family *Geminiviridae*, where the frequent recombinations that occur during mixed infections are one of the main sources of variability in this genus. To cite one case of heteroencapsidation, whitefly transmission of pseudorecombinants was demonstrated in a mixed infection between two bipartite viruses, *Tomato leaf curl Palampur virus* and *Tomato leaf curl New Delhi virus* in tomato and cucurbits (Kanakala et al. 2013). From an epidemiological point of view, these transcapsidation events occurring in the frame of a mixed viral infection can have a huge influence on the spread of a given virus, allowing it to be putatively transmitted by new vectors and arriving in new hosts and thus contributing to speeding up speciation events. In fact, recent taxonomic proposals, based on the similarities of viral polymerases (Wolf et al. 2018), argue in favor of evolutionary processes involving capture and switching of capsid genes, with mixed infections representing a key step to explain how these kinds of events could happen.

As mentioned previously, viruses are able to manipulate the host plant using different strategies that modify the vector biology, fitness, and behavior and consequently might have an impact on virus transmission and disease dynamics (Feres and Moreno 2009). This is known as indirect mutualism and confers advantages to the vector that would result in an increase of virus transmission and, consequently, spread (Li et al. 2014). Such modifications in the host mainly rely on nutritional changes and on a manipulation of the defense responses, making the plant more attractive and suitable for

the vector (Bak et al. 2017; Casteel and Falk 2016; Casteel et al. 2014, 2015; Li et al. 2014; Luan et al. 2014). This process is not random and the host alterations are strongly related to the transmission mode of each virus (Mauck 2016; Mauck et al. 2012). Briefly, plant viruses transmitted by insects are classified in two major groups, each one subdivided in two more groups, according to some parameters needed for acquisition, retention and inoculation. The time duration of those periods is related to the tissue tropism and the localization inside the host plant and to factors inside the insect. In the first mode of transmission, called “noncirculative,” the virus does not need to enter the internal cavity of the insect body crossing cellular barriers, and the virion particles are retained in the stylet or foregut region of the vector. If the retention of the virus in the vector is brief (seconds to minutes), viruses are classified in the subgroup showing a “nonpersistent” transmission mode. If it takes minutes to hours, they are classified as “semipersistent.” On the other hand, viruses that cross cellular barriers and reach the body cavity of the insect to end up in the salivary glands are named “circulative” or “persistent.” If they only circulate inside the vector, they are classified as “nonpropagative”; if there is virus multiplication inside the insect, the viruses are called “propagative” (Casteel and Falk 2016; Kaur et al. 2016; Mauck et al. 2018). Thus, a host plant that is modified to be attractive (by visual and olfactory clues), even if its quality as feed for a phytophagous insect is reduced, will be well adapted for a more efficient transmission of a nonpersistent virus: the vector is first attracted to the infected plant, but when it probes on it (and thus acquires the virus in a brief period of time), the insect does not receive enough reward to colonize and remain in the plant and then goes to another plant carrying the virus. On the other hand, viruses that are transmitted in a semipersistent or persistent manner will get benefit if they manipulate the host to promote long-term feeding, thus favoring acquisition of the virus (Mauck et al. 2012). These attraction-repulsion processes can be quite dynamic, and some works have described that feeding preferences and behavior of vectors could change after virus acquisition (Ingwell et al. 2012; Moreno-Delafuente et al. 2013; Rajabaskar et al. 2014). Collectively, all of these studies suggest that viruses might have been favored by natural selection to adopt strategies to enhance their transmissibility/dispersion in the context of mixed infections, using different means for that purpose, ranging from altering vector behavior via controlling vector interactions with their host plants to other mechanisms controlling transmissibility. The last column in the upper panel of Figure 1 illustrates that vectors can be essential parts of the payoff mechanisms achieved and favored during mixed infections.

IMPACT ON PLANTS

The host plant is the organism to be preserved in agricultural production; consequently, attention concerning the outcomes of mixed viral infections, considered as pathogenic attacks, has been mainly focused on synergistic or antagonistic responses. We previously mentioned such interactions in the previous sections, and an extensive catalog of known cases is provided in Tables 1 and 2, with comments primarily dedicated to provide information on how the different host plants are impacted by the simultaneous presence of the viruses mentioned. In this section, we will add some additional comments about a few cases that illustrate the wide variety of effects caused by the presence of multiple viruses in a host plant, particularly effects that go beyond the expected additive response to the individual viruses.

An example of host modification induced by a mixed infection can be found in grapes of plants infected with isolates of *Grapevine leafroll-associated virus 1*, *Grapevine virus A*, and *Rupestris stem pitting-associated virus* (Giribaldi et al. 2011). A proteomic analysis of samples from such mixed-infected plants revealed several changes in the host, with an expected major influence in the oxidative stress

responses (in the fruit skin), and in the cell structure (in the pulp). The oxidative stress responses can be induced by biotic stresses, including several pathogens (Demidchik 2015; Muthamilarasan and Prasad 2013). On the other hand, the alterations in cell structure might be linked to the cytoskeleton and the movement of viruses (Henry et al. 2006; Lucas 2006).

Another key element in the responses of plants toward viruses is RNA silencing or the post-transcriptional gene silencing pathways. RNA silencing is present in all eukaryotes and regulates many essential processes, such as chromatin modification, DNA methylation, and transposon activity, among many others, relying on activity of several types of the so-called “small RNAs.” In plants, RNA silencing is also one of the most important antiviral defense systems induced in response to both RNA and DNA viruses (Baulcombe 2004; Csorba et al. 2009; Vaucheret 2006; Wang et al. 2012). We have already anticipated in previous sections the importance of the mechanisms of RNA silencing for the outcome of mixed infections. In fact, likely all plant viruses encode at least one RNA silencing suppressor (Csorba et al. 2015); therefore, we should consider that at least two of them might be acting simultaneously during mixed infections. As indicated, the numerous cases of potyviruses contributing to synergistic responses during multiple infections have been associated with the strong activity of an HC-Pro as an RNA silencing suppressor (Pruss et al. 1997). Also within the same family, *Potyviridae*, a deep-sequencing study carried out in wheat showed that the synergistic mixed infection caused by the combination of the tritimovirus *Wheat streak mosaic virus* (WSMV) and the poacevirus *Triticum mosaic virus* (TriMV) resulted in major changes in the endogenous small RNA profile of the host plant, with an expected impact on the defense responses that differed between single and mixed infections (Tatineni et al. 2014).

The influence of the host plant on the outcome of viral mixed infections was mentioned previously, and there are some additional examples in Table 1. It has also been reported that in some synergistic viral combinations, the outcome varies depending on the host plant cultivar. For instance, the previously cited synergistic interaction between WSMV and TriMV induced different disease outcomes depending on the wheat cultivar (Tatineni et al. 2010). Other examples illustrate the influence of the cultivar in the outcome of the mixed infection, such as coinfections between (i) ToCV and TICV, (ii) PVX and PVY or *Tobacco etch virus*, and (iii) *Pepper huasteco virus* and *Pepper golden mosaic virus* (Gonzalez-Jara et al. 2004; Méndez-Lozano et al. 2003; Wintermantel et al. 2008). All of these cases likely reflect differences in the RNA silencing and antiviral responses of the host in a way that might play important roles not only in the establishment but also in the development of the disease. As a consequence, the management of viral diseases through control measures would need to consider the effects of mixed infections. Particularly worrisome would be the risk of compromising genetic resistance, as in the case of ToCV and TSWV discussed previously (García-Cano et al. 2006). Further investigations will be useful to determine whether other resistances can be compromised as well by the combined actions of viruses during mixed infections.

CONCLUDING REMARKS

In this review, we have pointed out several aspects related to mixed viral infections that support their great relevance not only for pathology but also for plant virus ecology and epidemiology. These two latter disciplines are partially related but deserve specific attention. When considering ecology, we refer to the interactions of the virus populations with the environment and its components, whereas the term “epidemiology” is more linked to the disease caused and how the virus is spread in the frame of complex associations between the viruses and the host plants, passing through the critically important participation of vectors (Jones 2014). Understanding why and how

pathogenic viruses can spread in a given ecosystem is the main challenge of epidemiology but the factors controlling the spread become more complex in a mixed infection scenario, and it is absolutely essential to understand them in order to build effective strategies to control the frequent diseases caused by multiple viral infections.

To prevent the negative effects of mixed infections, an important area of work is to develop methods or tools that interfere with transmission by vectors. As mentioned previously, the dispersion of the majority of plant viruses relies on the action of vectors as an efficient way to overcome the sessile nature of the plants on the one hand and to provide effective means to cross the strong cell wall

TABLE 2
Examples of mixed infections involving more than two viruses (or subviral/associated elements) simultaneously found in the same individual plant

Viruses ^a	Host ^b	Comments	References
Combinations of several (more than two) begomoviruses, such as <i>African cassava mosaic virus</i> (ACMV), <i>Cassava mosaic Madagascar virus</i> (CMMGV), <i>East African cassava mosaic virus</i> (EACMV), <i>East African cassava mosaic Kenya virus</i> (EACMKV), <i>East African cassava mosaic Malawi virus</i> (EACMMV), <i>East African cassava mosaic Zanzibar virus</i> (EACMZV), or <i>South African cassava mosaic virus</i> (SACMV) (all belonging to the genus <i>Begomovirus</i>)	Cassava (<i>Manihot esculenta</i>)	Causal agents of severe pandemics globally denominated as cassava mosaic disease. Synergism. Source of high recombination rates between genome components of the different viruses involved	Jacobson et al. (2018) and Rey and Vanderschuren (2017)
<i>Australian grapevine viroid</i> (AGV) (Apscaviroid), <i>Grapevine leafroll-associated virus-9</i> (GLRaV9) (Ampelovirus), <i>Grapevine rupestris stem pitting-associated virus</i> (GRSPaV) (Foveavirus), <i>Grapevine rupestris vein-feathering virus</i> (GRVfV) (Marafivirus), <i>Grapevine Syrah virus-1</i> (GSyV-1) (Marafivirus), <i>Grapevine yellow speckle viroid</i> (GYSVd) (Apscaviroid), <i>Hop stunt viroid</i> (HSVd) (Hostuviroid), or <i>Potato spindle tuber viroid</i> (PSTVd) (Pospiviroid)	Grapevine (<i>Vitis vinifera</i>)	Example of grapevine leafroll disease. Sequencing analysis of RNAs from a grapevine plant showing decline symptoms revealed the presence of up to seven different viruses and viroids in the same individual host	Al Rwahnih et al. (2009) and Naidu et al. (2015)
<i>Sweet potato chlorotic stunt virus</i> (SPCSV) (Crinivirus) and <i>Sweet potato feathery mottle virus</i> (SPFMV), together with at least one of the following viruses: <i>C-6 virus</i> (Carlavirus), <i>Cucumber mosaic virus</i> (CMV) (Cucumovirus), <i>Sweet potato chlorotic fleck virus</i> (SPCFV) (Carlavirus), <i>Sweet potato latent virus</i> (SPLV) (Potyvirus), <i>Sweet potato mild mottle virus</i> (SPMMV) (Ipomovirus), or <i>Sweet potato mild speckling virus</i> (SPMSV) (Potyvirus)	Sweet potato (<i>Ipomoea batatas</i>)	The presence of a third different virus in plants already affected with SPVD (SPCSV + SPFMV) increased the severity of symptoms. Synergism	Untiveros et al. (2007)
<i>Carrot red leaf virus</i> (CRLV) (Polerovirus), <i>Carrot mottle virus</i> (CMoV) (Umbravirus), <i>Carrot red leaf virus-associated RNA</i> (CtRLVaRNA)	Carrot (<i>Daucus carota</i>)	Causal agents of carrot motley dwarf disease. Synergism	Naseem et al. (2016), Vercruysse et al. (2000), and Watson et al. (1998)
<i>Ethiopian tobacco bushy top virus</i> (ETBTv) (Umbravirus), <i>Potato leafroll virus</i> (PLRV) (Polerovirus), <i>Satellite RNA of ETBTv</i> (SatRNA-E)	Tobacco (<i>Nicotiana tabacum</i>)	Recently emerged tobacco bushy top disease (TBD) in Ethiopia. Synergism	Abraham et al. (2014)
<i>Ipomoea vein mosaic virus</i> (IVMV) (Potyvirus), SPCSV (Crinivirus), SPFMV (Potyvirus), <i>Sweet potato virus G</i> (SPVG) (Potyvirus)	Sweet potato (<i>I. batatas</i>)	Mixed infections with two or three viruses. Synergism with SPCSV increasing the titers of potyviruses. No synergism observed between potyviruses	Kokkinos and Clark (2006)
PLRV (Polerovirus), <i>Potato virus A</i> (PVA) (Potyvirus), <i>Potato virus M</i> (PVM) (Carlavirus), <i>Potato virus S</i> (PVS) (Carlavirus), <i>Potato virus X</i> (PVX) (Potexvirus), <i>Potato virus Y</i> (PVY) (Potyvirus)	Potato (<i>Solanum tuberosum</i>)	Field surveys in Pakistan showing synergism for almost all of the mixed infections tested, frequently combining two or three viruses	Hameed et al. (2014)
SPCSV (Crinivirus), SPFMV (Potyvirus), SPMMV (Ipomovirus)	Sweet potato (<i>I. batatas</i>)	Synergism between SPCSV + SPFMV or SPMMV, and SPCSV + SPFMV + SPMMV. Neutralism between SPMMV and SPFMV	Mukasa et al. (2006)
<i>Tobacco bushy top virus</i> (TBTv) (Umbravirus), <i>Tobacco vein distorting virus</i> (TVDV) (Polerovirus), <i>Satellite RNA of TBTv</i> (Sat-TBTv), <i>Tobacco vein distorting virus associate RNA</i> (TVDVaRNA)	Tobacco (<i>N. tabacum</i>)	Causal agents of the TBD in several African countries and in China. Synergism and efficient aphid transmission reported in field conditions	Liu et al. (2014)

^a Viruses are listed in alphabetical order with their acronym and include the taxonomic adscription (genus) in parentheses.

^b Common names and scientific names are used to identify hosts.

and deliver viruses into the susceptible parts of the cell on the other hand. Usually, a viral infection begins with the specific interaction of the vector and the virus, mediated by proteins from both (Gutiérrez et al. 2013). As discussed in this review, in mixed infections, responses and changes can be seen both on vectors and viruses and can influence subsequent virus transmission. Thus, understanding in detail those modifications may be essential to interfere with key steps in virus–vector interactions and interrupt the viral cycle, limiting the spread of the infection (Whitfield and Rotenberg 2015).

The knowledge generated by the study of mixed viral infections could become a rich source of useful information to design effective control measures or even to engineer resistant plants against viruses. As mentioned in the section on the impact on viruses, one example of this latter strategy is represented by cross-protection, based on the antagonism between viruses and consisting of the activation of plant defenses (e.g., the RNA silencing mechanisms mentioned) by the first virus, thus preventing or alleviating the damaging infection by the second virus. This strategy has been successfully applied to protect plants of high economic interest, such as citrus, cucurbits, and papaya, through inoculation of plants with a mild strain of a virus, preventing the infection by a more virulent strain in the field (Ziebell and Carr 2010). However, we must recognize that the complexities of the interactions require more investigation. Indeed, when designing strategies to engineer resistant plants against viruses, it is worth taking into account the impact that possible mixed infections in nature can have. One relevant example reports the expression of an artificial microRNA targeting the viral RNA silencing suppressor HC-Pro of *Turnip mosaic virus* (TuMV) in *Arabidopsis thaliana* transgenic plants. High levels of resistance against the infection by the homologous virus were observed; however, if the plant was previously infected by *Tobacco rattle virus*, *Cauliflower mosaic virus*, or CMV, the resistance was lost and TuMV was able to infect the plant in the frame of a mixed infection with one of the other mentioned viruses (Martínez et al. 2013).

The relationship between viruses during mixed infections can have a clear impact on the evolution of each virus involved, as the competition for the host's resources directly influences the fitness of viruses. This competition can be specified at many levels, some of which are depicted schematically in Figure 2, and others are still waiting to be analyzed in depth. Among new aspects that might require attention are those occurring as a result of the different mechanisms of translation or the conflicting situation prompted by the different codon usages of the viral partners and the host (Adams and Antoniw 2004; Belalov and Lukashev 2013; Miras et al. 2017). As a result of these competitions, mixed infections must be considered as another key factor that influences virus evolution; indeed, recent studies have proposed models to predict the long-term evolution of viruses in the frame of mixed infections (Alizon et al. 2013; Elena et al. 2014; Escriu et al. 2003; Tollenaere et al. 2016). Virulence increases can be predicted as a result of the competitive advantage of virulent parasites, because the mixed infections are prone to alter the immune defense and cause phenotypic changes in the host. Thus, a higher virulence can be consequence of the competition for resources during a mixed infection (Choisy and de Roode 2010).

To confirm these predictions and incorporate the knowledge derived into strategies of virus control, more research will be highly desirable. The presence of more than one virus in the plant also makes possible genetic exchanges between them, and even the formation of new hybrid viral species containing parts from the genomes of the distinct viral species, through reassortments or recombinations (DaPalma et al. 2010). Eventually, speciation events might take place, thus contributing to the expansion of the global virome. Moreover, it seems that the choice of a given host by a given virus is not random, and it is driven by some still unknown mechanisms, which reinforces the importance of

multiple infections from an evolutionary and epidemiological point of view (Malpica et al. 2006).

Finally, recognizing the importance of mixed infections could also be instrumental for safer biotechnological exploitation of plant viruses (Pasin et al. 2019). For instance, we must take into account that when using a viral vector to express a protein or a valuable product in a susceptible plant that can be infected with another virus, we are dealing with a particular scenario of mixed infection and thus we must be aware of the possible consequences this can have on the pathosystem under study. On the other hand, the examples existing in nature where plants are able to tolerate, survive, and even thrive while supporting a multiple and highly variable virome, as in the case of sweet potatoes, deserve attention from researchers, since understanding how this particular plant can stand the multiple infections might provide hints for dealing with damage caused by viral pathogens in other crops. Genomic studies might give clues on key traits that could eventually provide wide-range tolerances to multiple stresses.

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