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Review

Facilitative and antagonistic interactions between plant viruses in mixed infections

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SUMMARY

Mixed infections of plant viruses are common in nature, and a number of important virus diseases of plants are the outcomes of interactions between causative agents. Multiple infections lead to a variety of intrahost virus–virus interactions, many of which may result in the generation of variants showing novel genetic features, and thus change the genetic structure of the viral population. Hence, virus–virus interactions in plants may be of crucial significance for the understanding of viral pathogenesis and evolution, and consequently for the development of efficient and stable control strategies. The interactions between plant viruses in mixed infections are generally categorized as synergistic or antagonistic. Moreover, mixtures of synergistic and antagonistic interactions, creating usually unpredictable biological and epidemiological consequences, are likely to occur in plants. The mechanisms of some of these are still unknown. This review aims to bring together the current knowledge on the most commonly occurring facilitative and antagonistic interactions between related or unrelated viruses infecting the same host plant. The best characterized implications of these interactions for virus–vector–host relationships are included. The terms 'synergism' and 'helper dependence' for facilitative virus–virus interactions, and 'cross-protection' and 'mutual exclusion' for antagonistic interactions, are applied in this article.

INTRODUCTION

Most attention in virology research has traditionally been given to properties of individual virus species, whereas comparatively little attention has been paid to within-host interactions between viruses or between viruses and microorganisms in multiple infections (Lidsky *et al*., 2009; Rentería-Canett *et al*., 2011). Meanwhile, accumulating evidence for ubiquitous viral infections in the plant and animal (including humans) kingdoms strongly suggests that mixed viral infections may be the rule rather than the exception in nature (DaPalma *et al*., 2010; Waner, 1994), which seems to be typical of the infections induced by parasites (Balmer *et al*., 2009).

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Plant viruses co-infecting the same host may generally interact in either a synergistic or an antagonistic way (e.g. García-Cano *et al*., 2006; Rentería-Canett *et al*., 2011; Untiveros *et al*., 2007). A synergistic interaction has a facilitative effect on both, or at least one, of the viral partners and is manifested by an increase in virus(es) replication in the host plant. A different situation occurs when one virus facilitates the transmission of another virus by vectors. This phenomenon naturally occurs in certain virus complexes (e.g. Murant, 1993; Syller, 2000, 2003, 2006) and is often termed 'helper dependence'. In contrast, in an antagonistic type of interaction, only one of the viruses is likely to be the beneficiary, and its presence and activity lower the fitness of the second virus. Furthermore, mixtures of synergistic and antagonistic virus–virus interactions, creating more or less predictable biological and epidemiological consequences, are likely to occur in plants (Zhang *et al*., 2001). To date, interference interactions have been concluded from population-level processes, such as increased or decreased plant fitness or the presence of shared vectors, rather than from laboratory experiments, mainly because of their complexity and the lack of suitable laboratory techniques.

Two pathways of multiple infection can be distinguished: co-infection and super-infection (Miralles *et al*., 2001; Saldaña *et al*., 2003). In co-infection, two or more viruses invade the host simultaneously or in a short interval of time. In super-infection, different viruses (strains) infect the host at different times. During natural virus outbreaks, the infection of hosts can occur in different scenarios. In the early phase of an epidemic, when potential hosts are highly available but viral density is low, a host usually becomes infected by a single virus at a time. However, when the epidemic continues to spread, more and more hosts become infected, thereby increasing the density of virus in the population. In the advanced phase of the epidemic, a chance for a newly released viral variant to encounter and invade an unoccupied host decreases, and the possibilities for mixed viral infections to occur increase with increasing time. However, irrespective of any differences in fitness between viral variants at the moment of the invasion, the primary virus has a numerical advantage for exploiting the limiting resources. A different situation arises when two homologous viruses enter a susceptible host cell. In this case, the environmental niche is available for both variants, neither of them gains a numerical advantage, and their further fate will largely

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Table 1 List of plant viruses addressed in this article.

Acronym	Species' name	Genus	Family
ALSV	*Apple latent spherical virus*	*Cheravirus*	Unassigned
BYDV-MAV	*Barley yellow dwarf virus-MAV*	*Luteovirus*	*Luteoviridae*
BYDV-PAV	*Barley yellow dwarf virus-PAV*	*Luteovirus*	*Luteoviridae*
BYMV	*Bean yellow mosaic virus*	*Potyvirus*	*Potyviridae*
CABMV	*Cowpea aphid-borne mosaic virus*	*Potyvirus*	*Potyviridae*
ClYVV	*Clover yellow vein virus*	*Potyvirus*	*Potyviridae*
CMV	*Cucumber mosaic virus*	*Cucumovirus*	*Bromoviridae*
CTV	*Citrus tristeza virus*	*Closterovirus*	*Closteroviridae*
GRAV	*Groundnut rosette assistor virus*	*Luteovirus*	*Luteoviridae*
GRV	*Groundnut rosette virus*	*Umbravirus*	Unassigned
MCMV	*Maize chlorotic mottle virus*	*Machlomovirus*	*Tombusviridae*
PAMV	*Potato aucuba mosaic virus*	*Potexvirus*	*Flexiviridae*
PEMV-1	*Pea enation mosaic virus-1*	*Enamovirus*	*Luteoviridae*
PEMV-2	*Pea enation mosaic virus-2*	*Umbravirus*	Unassigned
PepGMV	*Pepper golden mosaic virus*	*Begomovirus*	*Geminiviridae*
PepMoV	*Pepper mottle virus*	*Potyvirus*	*Potyviridae*
PHV	*Pepper huasteco virus*	*Begomovirus*	*Geminiviridae*
PLRV	*Potato leafroll virus*	*Polerovirus*	*Luteoviridae*
PPV	*Plum pox virus*	*Potyvirus*	*Potyviridae*
PVS	*Potato virus S*	*Carlavirus*	*Betaflexiviridae*
PVX	*Potato virus X*	*Potexvirus*	*Flexiviridae*
PVY	*Potato virus Y*	*Potyvirus*	*Potyviridae*
SPCSV	*Sweet potato chlorotic stunt virus*	*Crinivirus*	*Closteroviridae*
SPFMV	*Sweet potato feathery mottle virus*	*Potyvirus*	*Potyviridae*
TEV	*Tobacco etch virus*	*Potyvirus*	*Potyviridae*
TICV	*Tomato infectious chlorosis virus*	*Crinivirus*	*Closteroviridae*
TMV	*Tobacco mosaic virus*	*Tobamovirus*	*Virgaviridae*
ToCV	*Tomato chlorosis virus*	*Crinivirus*	*Closteroviridae*
TriMV	*Triticum mosaic virus*	*Poacevirus*	*Potyviridae*
TSWV	*Tomato spotted wilt virus*	*Tospovirus*	*Bunyaviridae*
TuMV	*Turnip mosaic virus*	*Potyvirus*	*Potyviridae*
TVMV	*Tobacco vein mottling virus*	*Potyvirus*	*Potyviridae*
WSMV	*Wheat streak mosaic virus*	*Tritimovirus*	*Potyviridae*
ZYMV	*Zucchini yellow mosaic virus*	*Potyvirus*	*Potyviridae*

depend on their relative fitnesses (Miralles *et al*., 2001; Saldaña *et al*., 2003).

Mixed infections lead to a great variety of within-host virus–virus interactions. Some may result in the generation of variants showing novel genetic features, and thus change the genetic structure of the viral population. Hence, interactions among viruses may be crucial for the understanding of viral pathogenesis and evolution (Read and Taylor, 2001), and consequently for the development of efficient and stable control strategies (García-Arenal *et al*., 2003; Rentería-Canett *et al*., 2011). As emphasized by Rentería-Canett *et al*. (2011), the number of reports on mixed infection has increased recently, providing valuable knowledge that may be useful in controlling complex diseases.

The aim of this article is to bring together the current knowledge on the most commonly occurring facilitative and antagonistic interactions between unrelated or related viruses infecting the same host plant, and on the best-characterized implications of these interactions for virus–vector–host relationships. The terms 'synergism' and 'helper dependence' for facilitative interactions, and 'cross-protection' and 'mutual exclusion' for antagonistic interactions, are applied. A list of plant viruses addressed in this review is shown in Table 1.

FACILITATIVE INTERACTIONS

Synergism

This phenomenon, also termed 'synergy', refers to a situation in which mixed infection of a plant with two or more viruses results in increased multiplication of one or both viruses, and viral partners interacting with each other induce symptoms more severe than would be expected if they interacted in an additive manner (e.g. García-Cano *et al*., 2006; Pruss *et al*., 1997; Untiveros *et al*., 2007; Vance, 1991; Zhang *et al*., 2001), as shown in Fig. 1.

Numerous synergistic interactions have been described, the best characterized of which are those involving a potyvirus (genus *Potyvirus*, family *Potyviridae*) as one of the viral partners. The classical example is the interaction between *Potato virus Y* (PVY) and *Potato virus X* (PVX) in tobacco plants (*Nicotiana tabacum*), resulting in an enhancement of disease symptoms and in an up to

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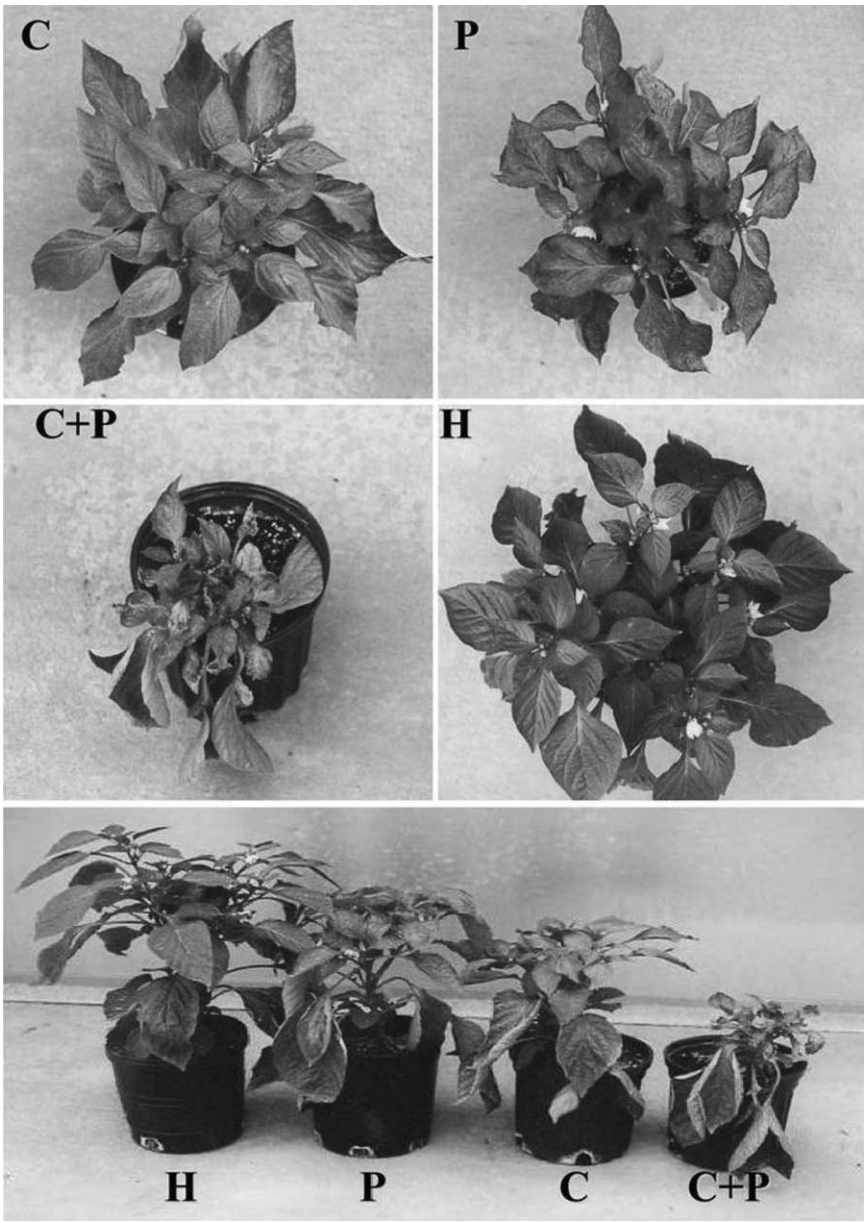

Fig. 1 Symptoms induced in pepper plants by *Cucumber mosaic virus* (C), *Pepper mottle virus* (P) and the combined infection of C + P at 21 days post-inoculation. A comparable mock-inoculated plant is included (H). Figure and text taken from Murphy and Bowen (2006) with kind permission of the copyright owner The American Phytopathological Society.

10-fold increase in the titre of PVX compared with single infections (Rochow and Ross, 1955; Vance, 1991). In contrast, no marked increase in the accumulation of PVX was recorded in *N. benthamiana* plants co-infected with PVY, *Tobacco etch virus* (TEV) or *Plum pox virus* (PPV), despite the severe reaction leading to systemic necrosis of leaves and stems, and finally plant death (González-Jara *et al*., 2004, 2005). These observations indicate that the enhancement of disease symptoms is not simply a result of the increase in PVX accumulation in plants. Hence, it has been suggested that the synergy pattern between PVX and a potyvirus is host dependent (González-Jara *et al*., 2004). Host-dependent differences in virus accumulation and alteration of accumulation patterns during co-infection, compared with single infection, have also been reported for *Tomato chlorosis virus* (ToCV) and *Tomato infectious chlorosis virus* (TICV) (Wintermantel *et al*., 2008). In doubly infected *N. benthamiana* plants, TICV titres increased and ToCV titres decreased, when compared with concentrations in singly infected plants, whereas, in co-infected *Physalis wrightii* plants, titres of both viruses decreased. The pattern of TICV–ToCV–host interactions suggests the existence of differences between the two viruses in adaptation to different hosts, and these differences may finally translate into competitiveness of each virus in doubly infected hosts. Host-dependent alteration of the symptoms has been reported for plants co-infected with *Pepper huasteco virus* (PHV) and *Pepper golden mosaic virus* (PepGMV), because synergism was observed in tobacco and *N. benthamiana*, whereas

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antagonism was detected in pepper (Méndez-Lozano *et al*., 2003). A synergy pattern may not only depend on the host species, but also on the host cultivar, as recently reported for three wheat cultivars co-infected with *Wheat streak mosaic virus* (WSMV) and *Triticum mosaic virus* (TriMV) (Tatineni *et al*., 2010).

Interestingly, in most of the reported cases, the concentration of the potyvirus, such as PVY, TEV, *Tobacco vein mottling virus* (TVMV), *Zucchini yellow mosaic virus* (ZYMV), *Pepper mottle virus* (PepMoV) and *Cowpea aphid-borne mosaic virus* (CABMV), involved in a synergistic interaction with a heterologous virus, remained unaffected or slightly decreased, whereas the accumulation of the nonpotyvirus increased (Murphy and Bowen, 2006; Pruss *et al*., 1997; Taiwo *et al*., 2007; Vance *et al*., 1995; Zeng *et al*., 2007; and references cited therein). However, in several instances, the reverse relationship was observed, in which the concentration of the potyvirus increased, and that of the nonpotyviral partner remained constant. This was the case for PVY in *Solanum brevidens* co-infected with *Tobacco mosaic virus* (TMV) (Valkonen, 1992), and of *Sweet potato feathery mottle virus* (SPFMV) in sweet potato in mixed infection with *Sweet potato chlorotic stunt virus* (SPCSV) (Cuellar *et al*., 2008; Karyeija *et al*., 2000; Mukasa *et al*., 2006). It has also been demonstrated that SPCSV, but not SPFMV, can cause synergistic diseases in sweet potato with members of the virus genera *Potyvirus* and *Ipomovirus* (family *Potyviridae*), *Cucumovirus* (family *Bromoviridae*) and *Carlavirus* (family *Betaflexiviridae*) (Untiveros *et al*., 2007). Surprisingly, both the tritimovirus WSMV and the machlomovirus *Maize chlorotic mottle virus* (MCMV) seemed to profit from mixed infections in maize plants (Scheets, 1998; Stenger *et al*., 2007). Such a synergy pattern was also observed with WSMV and TriMV in wheat plants (Tatineni *et al*., 2010). In addition, the interaction between PVY and *Cucumber mosaic virus* (CMV) appeared to be beneficial for both pathogens, but this mutualistic relationship was found to be peculiar to tomato (Mascia *et al*., 2010). The role of a potyvirus-encoded helper component in the PVY–CMV interaction is described below.

Synergistic interactions are known to be produced predominantly by unrelated viruses that infect the same host cells. If so, the above-mentioned facilitative effect of the phloem-limited crinivirus SPCSV on the potyvirus SPFMV invading nonphloem tissue is surprising (Karyeija *et al*., 2000). The molecular mechanisms of this synergism are not known, but the authors proposed two hypotheses that remain to be verified. One assumes that certain proteins encoded by SPCSV are transported from the phloem to other tissues, enhancing the multiplication of SPFMV and hence mediating the synergism. According to another hypothesis, the resistance mechanism of sweet potato strongly inhibits the multiplication of SPFMV, but SPCSV suppresses its activity (Karyeija *et al*., 2000). Another unexpected synergistic interaction has been reported for the phloem-limited *Potato leafroll virus* (PLRV) and PVY (Srinivasan and Alvarez, 2007). Potato plants with mixed infections of PVY and PLRV showed more severe symptoms than plants infected by either virus alone, but no essential differences in the virus titre were recorded between doubly and singly infected plants.

It should be emphasized that, apart from those for unrelated viruses, synergistic interactions have also been reported for more or less closely related viruses, such as several *Begomovirus* species (Chakraborty *et al*., 2008; Méndez-Lozano *et al*., 2003; Rentería-Canett *et al*., 2011; Sufrin-Ringwald and Lapidot, 2011), two *Crinivirus* species (Wintermantel *et al*., 2008), and two viruses belonging to the family *Potyviridae*, although to different genera (Tatineni *et al*., 2010).

Different synergy patterns can be viewed as the outcomes of battles between viral and host genes during double infections of different hosts. A synergistic plant response to mixed infection involving a potyvirus is mediated by the expression of potyviral helper component-proteinase (HC-Pro) (Stenger *et al*., 2007). HC-Pro is a multifunctional protein controlling diverse processes important for the viral cycle, including the suppression of post-transcriptional gene silencing (PTGS) (reviewed by Maia *et al*., 1996; Syller, 2006; Urcuqui-Inchima *et al*., 2001), which is a naturally occurring plant defence mechanism that uses small interfering RNAs (siRNAs) against invading nucleic acids, such as viruses (Matzke *et al*., 2001; Mlotshwa *et al*., 2008). The PVX–potyvirus synergy has been reported to be mediated by the P1/HC-Pro sequence of HC-Pro (Shi *et al*., 1997; Vance *et al*., 1995). The same potyviral sequence has been shown to exacerbate the pathogenicity and accumulation of two other heterologous viruses: CMV and TMV (Pruss *et al*., 1997). HC-Pro mediates synergistic interactions by suppression of PTGS (González-Jara *et al*., 2005; Stenger *et al*., 2007; and references cited therein). However, some findings suggest that disease induced by potyvirus–PVX synergism may involve interference with multiple RNA silencing pathways (González-Jara *et al*., 2005). It was found, among other things, that, in addition to its function as the suppressor of PTGS, the HC-Pro of *Turnip mosaic virus* (TuMV) was able to interfere with micro-RNA (miRNA)-guided mRNA cleavage (Kasschau *et al*., 2003), known as another manifestation of RNA silencing (Burgyán, 2006). More recently, the HC-Pro encoded by PVY has been reported to be a much more efficient enhancer of CMV (or a recombinant CMV vector) concentrations in mixed infected *N. benthamiana* plants than the HC-Pros encoded by TuMV or *Clover yellow vein virus* (ClYVV) (Fukuzawa *et al*., 2010). The PVY HC-Pro expressed in transgenic plants was sufficient to cancel the cycling pattern of the CMV titre, resulting in increased levels of overall CMV accumulation. Moreover, the levels of CMV were much higher in the HC-Pro transgenic plants than in plants mixed infected with CMV and PVY. Most probably, the CMV–PVY synergy is the effect of RNA silencing by the PVY HC-Pro, but the involvement of the CMV 3a movement protein is also possible (Fukuzawa *et al*., 2010).

Apart from the potyviral HC-Pro, certain proteins encoded by other viruses are also capable of suppressing RNA silencing (Niehl

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and Heinlein, 2011; Qu and Morris, 2005; Roth *et al*., 2004; Voinnet, 2005; Wei *et al*., 2001), thereby determining the synergy pattern. Different viral suppressors act on different stages in PTGS. For example, the p25 movement protein of PVX, one of the best-studied suppressors of RNA silencing, has been reported to prevent the accumulation of the mobile silencing signal by interfering with the cellular RNA-directed RNA polymerase branch of the pathway (Voinnet *et al*., 2000). More recent studies have shown that the counter-defence role of p25 is through the degradation of Argonaute (Ago) proteins via the proteasome pathway (Chiu *et al*., 2010). Interestingly, the HC-Pro encoded by WSMV, the above-mentioned tritimovirus distantly related to potyviruses (Stenger *et al*., 1998), has been proven to be dispensable for inducing synergistic effects in maize plants doubly infected with WSMV and MCMV (Stenger *et al*., 2007). Seemingly, WSMV HC-Pro does not act as a suppressor of PTGS, which implies that PTGS suppression and disease synergism are mediated by protein(s) encoded by other WSMV gene(s) (Stenger *et al*., 2007).

The ability of plant viruses to cause disease synergism in crop plants has biological, epidemiological and economic implications. The increased multiplication of one or both interacting viral partners may have modifying effects on the virus host range and rate of vector transmission (Elena, 2011). The former relationship can be exemplified by the breakage of resistance to *Tomato spotted wilt virus* (TSWV) in tomato plants co-infected with the crinivirus ToCV (García-Cano *et al*., 2006), to CMV in cucumber plants co-infected with ZYMV (Wang *et al*., 2004) and to a number of viruses in sweet potato simultaneously infected with the crinivirus SPCSV (Karyeija *et al*., 2000; Mukasa *et al*., 2006; Untiveros *et al*., 2007). When the resistance to systemic infection by a given virus or strain is overcome, the plant changes from a nonhost to a host, thereby extending the virus host range. This is, of course, a very simplistic explanation, because systemic infection of the host plant is a complex process that involves several steps preceding a successful invasion of the plant by a virus: infection, replication, cell-to-cell movement and long-distance movement (Dawson and Hilf, 1992). In turn, the plant must possess specific protein components that interact with viral gene products at each step during the infection process The loss of resistance to a viral pathogen in crop plants can be further considered in terms of the economic consequences of disease synergism.

The effects of synergistic interactions during natural mixed infections on the rate of vector transmission deserve special attention, as they may have serious ecological and epidemiological consequences. The transmission rate is usually estimated as the percentage of plants that become infected following inoculation of viral particles by vectors that have fed previously on infected plants. Increased concentration of one or both viruses in double infections may result in increased vector transmission that, in general, is positively correlated with virus accumulation (Froissart *et al*., 2010), as has been demonstrated, for example, for aphid-transmitted viruses, irrespective of the transmission manner (nonpersistent or persistent) (e.g. Barker and Woodford, 1992; De Bokx *et al*., 1978; Gray *et al*., 1991; Pereira *et al*., 1989). Indeed, the transmission efficiency of the criniviruses ToCV and TICV by whiteflies (Homoptera: Aleyrodidae) corresponded to virus concentration in the host in both single and double infections (Wintermantel *et al*., 2008). In addition, the possibility of an increased infection rate by mite (Acarina: Tarsonemidae) transmissions from plants doubly infected by WSMV and TriMV (family *Potyviridae*) than from singly infected plants has recently been indicated (Tatineni *et al*., 2010). Moreover, it is relevant that, in double infections, both or at least one of the viruses may not only accumulate to a largely increased level, but may also broaden virus distribution in the host, thereby increasing virus availability for feeding vectors (Mascia *et al*., 2010). It has also been reported that mixed viral infections can affect the biology and preference of virus vectors. The fecundity of *Myzus persicae* and *Macrosiphum euphorbiae* (Homoptera: Aphididae), the efficient vectors of PLRV and PVY, was significantly higher on plants doubly infected with these viruses than on plants singly infected with PVY, but not PLRV (Srinivasan and Alvarez, 2007). As postulated by the authors, such an outcome could be the result of inhibited phloem transport and increased accumulation of sugars and amino acids in the phloem in mixed infected plants and PLRV-infected plants compared with PVY-infected plants and noninfected plants. Furthermore, both aphid species preferentially settled on doubly infected plants. It is probable that the visual and/or olfactory stimuli emitted by mixed infected plants were more attractive to aphids than were the stimuli emitted by singly infected or noninfected plants (Srinivasan and Alvarez, 2007; and references cited therein). Plant-mediated interactions between PVY/PLRV and aphid vectors may have significant and far-reaching implications for disease epidemiology, as the two viruses often occur in mixed infections (e.g. Chatzivassiliou *et al*., 2008; Srinivasan and Alvarez, 2007).

Synergistic interactions enhancing virus pathogenicity may increase plant damage, especially to susceptible cultivars, and thereby increase yield loss (e.g. Kareem and Taiwo, 2007; Malik *et al*., 2010; Murphy and Bowen, 2006; Tatineni *et al*., 2010; Zhang *et al*., 2001; and references cited therein). Some of the naturally occurring double or triple infections have been reported to cause devastating synergy diseases, such as maize lethal necrosis disease (Scheets, 1998), cassava mosaic disease (Pita *et al*., 2001) and sweet potato virus disease (Mukasa *et al*., 2006; Untiveros *et al*., 2007), often leading to the premature death of plants and, consequently, to substantial yield losses. The protection of crops against epidemiological outbreaks of the diseases should include the planting of resistant cultivars, the prevention of early infections facilitating viral synergy, the production of virus-free seed material and the control of virus vectors (Kareem and Taiwo, 2007; Tatineni *et al*., 2010).

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Helper dependence

Some viruses are dependent on other viruses for certain phases of their life cycles. Consequently, the former are termed 'dependent viruses' and the latter are termed 'helpers'. In a 'helper-dependent' virus complex, there is a complete unilateral facilitation of the dependent virus by the helper (Zhang *et al*., 2000). Helper dependence has been demonstrated in various virus–virus combinations among human, animal and plant viruses (DaPalma *et al*., 2010).

Despite the differences in sequences and genome organization, taxonomically distinct species of plant viruses have frequently been demonstrated to exhibit complementary functions in virus cell-to-cell and long-distance transport (e.g. Ajjikuttira *et al*., 2005; Rao *et al*., 1998; Ryang *et al*., 2004; and references cited therein). Complementation can be defined as the process by which the function affected by a mutation is provided *in trans* by fully competent genotypes in multiple-infected cells (Fraile *et al*., 2008). The efficiency of complementation of defective mutants by plant viruses has been found to be high under both experimental and field conditions. The phenomenon may result in host range extension and modified tissue tropism, and may also be relevant for the management of viral diseases if the complemented deleterious mutation is linked to other functions affecting the pathogenicity or epidemiology of the virus (Fraile *et al*., 2008).

Importantly, the helper dependence among plant viruses refers to specific associations with invertebrate animals that most viruses have evolved to be transmitted from plant to plant (e.g. Syller, 2000). There are numerous examples of interactions between vector-transmissible viruses and viruses that are unable to associate for transmission with any invertebrate. The latter are best exemplified by the species assigned to *Umbravirus*, a genus comprising viruses that lack genetic information for a capsid protein, and thereby for vector transmission, and also exhibit other specific features. Both indirect associations of umbraviruses with aphid vectors via helper viruses and the biological and molecular features of these extraordinary viruses have been comprehensively reviewed elsewhere (Syller, 2003; Taliansky and Robinson, 2003). Thus, only the virus properties relevant to the major topic of this review will be recalled here, and updated.

Unlike many other plant viruses, an umbravirus cannot be transmitted by aphids, but it becomes aphid transmissible if the host plant is co-infected with a suitable virus from the family *Luteoviridae* that acts as the helper. Under natural conditions, luteovirids are transmitted only by aphids, in a circulative non-propagative manner, and umbraviruses have been found only in plants co-infected with luteovirids. In mixed infection, the umbraviral RNA can be encapsidated by the capsid protein of the helper virus. The virion so assembled is readily acquired by a luteovirid's vector feeding on the infected plant and transmitted in a circulative fashion to the next plant (for references, see Syller, 2003). In reality, the umbravirus–luteovirid interaction is more complex, and its best studied outcome is groundnut rosette disease (GRD), the most destructive disease of groundnut (*Arachis hypogaea*) in sub-Saharan Africa. It is caused by the complex consisting of the umbravirus *Groundnut rosette virus* (GRV), its luteovirid partner *Groundnut rosette assistor virus* (GRAV) and the satellite RNA (satRNA) of GRV. Both, GRAV and GRV replicate independently in the host and move efficiently from cell to cell and over long distances, whereas satRNA depends on GRV RNA for its multiplication. In nature, all three GRV agents must be transmitted together to produce the disease, thereby maintaining the mixed infection. For encapsidation by GRAV capsid protein and aphid transmission, GRV depends on satRNA, whereas both, in turn, must be packaged into the capsid protein of GRAV to be transferred by aphids to the next plant.

It should be mentioned that, in most cases, umbraviruses and their helpers are infectious as independent entities, with the exception of the enamovirus *Pea enation mosaic virus-1* (PEMV-1), which is unable to move within the host unless assisted by the umbravirus PEMV-2 (for references, see Syller, 2003). Groundnut plants affected by GRD develop severe disease symptoms, whereas infection with either GRV or GRAV alone produces no overt symptoms. Mathematical models employed to analyse plant infection by helper-dependent virus complexes, including GRD, allowed Zhang *et al*. (2000) to conclude that, if the helper virus and host are mutually adapted to each other, the dependent virus that shares the host has an increased chance of evolving to constitute a helper-dependent virus complex and is more likely to survive subsequent evolutionary changes. This could explain why, for example, infection with GRAV alone causes no or minor damage to *A. hypogaea* plants, whereas co-infection with the GRV–satRNA complex results in very serious damage to this host (Zhang *et al*., 2000). However, more recent studies have shown that single infection of groundnut with GRAV markedly affects plant growth in some genotypes and causes yield losses reaching 52%, although the infected plants only develop mild yellowing of foliage (Naidu and Kimmins, 2007).

A different helper strategy, based on the encoding of HC-Pro, is used by potyviruses (e.g. Brault *et al*., 2010; Froissart *et al*., 2002; Maia *et al*., 1996; Pirone and Blanc, 1996; Syller, 2006). HC-Pro was hypothesized by Govier and Kassanis (1974) to bind both to virions and to the cuticular lining of aphid mouthparts (a model termed the 'bridge hypothesis'; Pirone and Blanc, 1996), thus retaining the virions within the food canal of the aphid stylets (Fig. 2). The attachment of a potyvirus to a suitable HC-Pro requires an association between the HC-Pro motifs and specific sites on the virion. Such a short-term reversible binding is sufficient for successful virus transmission from one plant to another.

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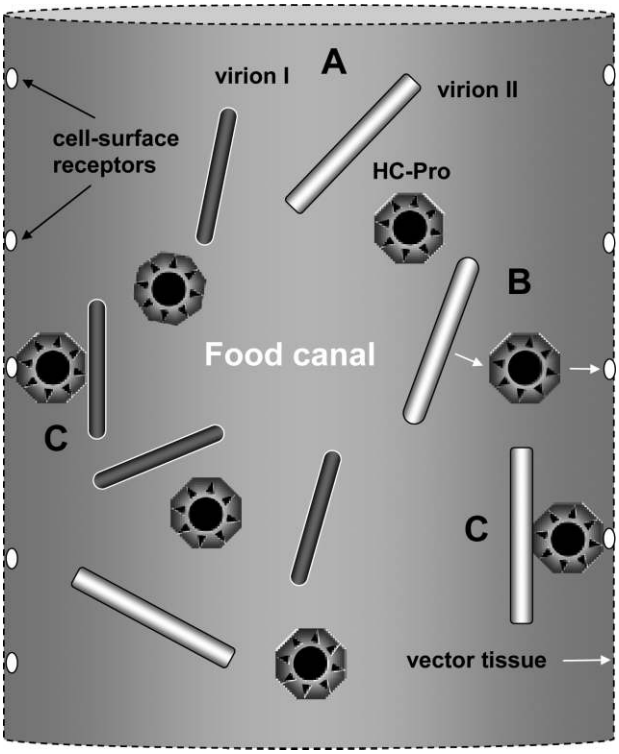

Fig. 2 Model illustrating the function of helper component-proteinase (HC-Pro) in nonpersistent transmission of viruses by aphid vectors. HC-Pro acts as a reversible 'bridge' in attaching the virion to the cuticle of the maxillary food canal and foregut of aphid vectors. (A) Free virions of two different viruses (virion I and virion II) and HC-Pro molecules homologous to one of the viruses present in plant sap acquired by the aphid. (B) The process of the linking of HC-Pro to a specific receptor on the vector tissue, followed by binding of virion I or virion II to HC-Pro. (C) HC-Pro-virion (I or II) complex bound to the vector stylet.

In mixed infection, a biologically active HC-Pro of one virus can facilitate aphid transmission of the second virus. An HC-Pro protein encoded by a helper potyvirus can mediate the transmission of an HC-Pro-deficient and thus nontransmissible isolate of the same species, as well as of related or even unrelated viruses (Fig. 2), *Potato aucuba mosaic virus* (PAMV) being the best documented example (Baulcombe *et al*., 1993; Kassanis, 1961; Kassanis and Govier, 1971a, b; Manoussopoulos, 2001). However, it was found that mixed infection with a potyvirus is not a prerequisite for aphid transmission of PAMV, as this virus appeared to be transmissible from singly infected plants, provided that aphids had previously fed on a plant infected with a potyvirus (Kassanis and Govier, 1971a). A consequence of the sequential acquisition is that HC-Pro acquired by the aphid can assist the transmission of virions located in the same cell, in other cells, or even in another host plant that is subsequently probed by the vector. For this phenomenon, the term 'HC-transcomplementation' has been proposed (Froissart *et al*., 2002).

ANTAGONISTIC INTERACTIONS

Cross-protection

This type of competitive virus–virus interaction, often termed 'super-infection exclusion' or 'homologous interference', occurs when a previous infection with one (protecting) virus prevents or interferes with subsequent infection by a homologous virus (DaPalma *et al*., 2010; Gal-On and Shiboleth, 2005; González-Jara *et al*., 2009; Zhang and Holt, 2001; Ziebell and Carr, 2010). In the past, this phenomenon was utilized to establish virus relationships, as only related viruses would show the response (Zaitlin and Palukaitis, 2000). At present, the availability of serological- and nucleic acid-based techniques makes this method comparatively much less attractive and useful (Ziebell and Carr, 2010).

The two viruses can replicate and move cell-to-cell and long distance as independent entities. However, when infected with the protecting virus, the host plant becomes resistant to super-infection with a related challenging virus, or disease symptoms induced by the latter are suppressed. In this respect, cross-protection resembles the 'vaccine' concept in human and veterinary medicine. Several mechanisms have been proposed for the phenomenon (e.g. Gal-On and Shiboleth, 2005; Lecoq and Raccah, 2001; Urban *et al*., 1990; Ziebell, 2008; Ziebell and Carr, 2010). These include, among others, a prevention of the disassembly of the challenging virus by the expression of the coat protein of the protecting virus (Powell-Abel *et al*., 1986; Sherwood and Fulton, 1982) and the induction of RNA silencing by the protecting virus, presumably by sequence-specific degradation of the challenging virus RNA (Fagoaga *et al*., 2006; Ratcliff *et al*., 1997, 1999). As considered by Sarika *et al*. (2010), the strongest evidence is for the former concept. However, the hindrance of virus uncoating may not be the only mechanism of cross-protection, as there is also evidence that the coat protein may interfere with the process of replication of the challenging virus (Sarika *et al*., 2010).

From an evolutionary standpoint, super-infection exclusion can appear to be beneficial to a newly produced viral variant by favouring its entry into uninfected rather than previously infected host cells, thereby promoting virus dissemination. Moreover, a primary virus successfully infecting a cell would be protected from a competing, super-infecting virus. Therefore, one can speculate that super-infection exclusion plays a significant role in maintaining the genetic diversity of a virus population because it allows the replication of variants that range in fitness.

Since the discovery of the phenomenon by McKinney in 1929 (after Zaitlin and Palukaitis, 2000), cross-protection has been applied with greater or lesser success to protect cultivated plants against detrimental viral diseases (e.g. Freitas and Rezende, 2008; Fulton, 1986; Gal-On and Shiboleth, 2005; Hanssen *et al*., 2010; Lecoq and Raccah, 2001; Nakazono-Nagaoka *et al*., 2009; Singh and Singh, 1995; Walkey, 1992). Success in virus control by

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this method depends on whether the attenuated virus isolate can invade the plant and displace the virulent virus (Zhang and Holt, 2001). In practice, to invade the host, a mild or attenuated isolate must be artificially inoculated to the plant as a protective means against infection by virus isolates causing severe disease. This procedure is applicable in the glasshouse, but places a limit on the application of cross-protection under field conditions.

However, some vector-borne viruses occur in the field in numerous strains, many of which cause mild symptoms and may naturally play a role in protecting viruses. One of the best-studied examples is cross-protection among isolates of *Citrus tristeza virus* (CTV), the aphid-transmitted virus causing economically important diseases of citrus worldwide (Bar-Joseph *et al*., 1989). Mild isolates of CTV proved to protect infected citrus plants against the effects of virulent strains but, in many varieties and growing areas, the lack of effective protecting isolates has greatly hindered the use of cross-protection (Folimonova *et al*., 2010; and references cited therein). Recent trials to elucidate why some CTV isolates are effective in preventing super-infection and others are not showed that super-infection exclusion occurred only between isolates of the same strain and not between isolates of different strains (Folimonova *et al*., 2010). Based on their results, the authors concluded that super-infection exclusion by CTV cannot be explained on the basis of RNA silencing, thereby implying the existence of a novel mechanism that remains to be determined.

The phenomenon of cross-protection was hypothesized by Power (1996) to have been involved in the puzzling change in the predominant strain of *Barley yellow dwarf virus* (BYDV) from MAV to PAV in spring oat in New York State in the years 1957–1976, reported by Rochow (1979). As typical luteoviruses (D'Arcy and Domier, 2005), PAV and MAV are only transmitted by aphids in a circulative nonpropagative manner. Virus–vector relationships may have played a crucial role in the MAV → PAV substitution, because vector abundance, the production of winged aphid morphs, vector preference behaviour and vector movement presumably favoured the transmission of PAV rather than MAV (Power, 1996). According to the mathematical model proposed by Zhang and Holt (2001), cross-protection between MAV and PAV led to a faster relative increase for PAV, which had a larger transmission rate during each growing season. However, the putative role of cross-protection in the exclusion of MAV by PAV has been questioned by Caciagli (2004), who otherwise found Zhang and Holt's model feasible with regard to the explanation of the MAV → PAV shift in terms of the prevalence of the more competitive strain PAV. He postulated that the 20-year period analysed by Rochow (1979), and re-analysed by Power (1996), Zhang and Holt (2001) and Amaku *et al*. (2010), is part of the long-term competition between the two BYDV strains.

Mutual exclusion

The literature on mutual exclusion (also termed 'mutual suppression' or 'mutual competitive suppression') is quite rich for human parasite infections (e.g. Balmer *et al*., 2009; Pepin *et al*., 2008), but scarce for intrahost interactions between viruses in plants. For the phenomenon to occur, two or more viruses must infect a host at the same time. In the 1960s, the phenomenon was reported for three strains of aster yellows phytoplasma, once thought to be a virus (Freitag, 1964). Mutual exclusion was also observed in oat plants simultaneously inoculated in the early growth stage with three strains of BYDV (Jedlinski and Brown, 1965). It was exhibited by mild symptoms developed by plants soon after inoculation, followed by complete recovery of the plants, from which no virus could be detected.

The mechanism for mutual exclusion is still obscure. It has recently been proposed that, based on the current knowledge on interactions between viruses and host plants, a plant may be viewed as a spatially structured environment for plant viruses (Elena *et al*., 2011). The evidence for spatial exclusion of closely related viruses is accumulating. When *N. benthamiana* plants were doubly inoculated with cDNA clones of the potyviruses PPV, TVMV and ClYVV expressing green and red fluorescent proteins (GFP and RFP), or with identical but differently labelled potyviruses (e.g. PPV-GFP and PPV-RFP), the two viral populations competed with each other during the colonization of epidermal cells (Dietrich and Maiss, 2003). Both fluorescence signals were only visible in some cells at the border of two neighbouring, differently coloured cell clusters. In addition, Takeshita *et al*. (2004) reported that two strains of CMV, although belonging to different CMV subgroups, did not colonize the same cells in co-infected cowpea plants. Similar results were obtained using *Apple latent spherical virus* (ALSV) to investigate the distribution of identical, but expressing yellow vs. cyan fluorescent proteins (ALSV-YFP vs. ALSV-CFP), virus populations in co-infected *Chenopodium quinoa* plants (Takahashi *et al*., 2007) (Fig. 3). Differently labelled ALSV populations were always distributed separately in both inoculated and upper uninoculated leaves. Moreover, when *C. quinoa* leaves were first inoculated with ALSV-CFP and then the same leaves were re-inoculated with ALSV-YFP, the latter virus infected only the tissues in which ALSV-CFP infection had not been established. The phenomenon of spatial separation was also observed in *N. benthamiana* leaves simultaneously inoculated with *Bean yellow mosaic virus* (BYMV) differently labelled with YFP and CFP (Takahashi *et al*., 2007).

Undoubtedly, spatial separation generates a specific bottleneck during virus infection, preventing extensive multiple infection of plant cells by several genomes of the viral population (Monsion *et al*., 2008). The key parameter for the evaluation of the kinetics and progress of multiple infection is the multiplicity of infection (MOI), i.e. the number of viral genomes that enter and effectively

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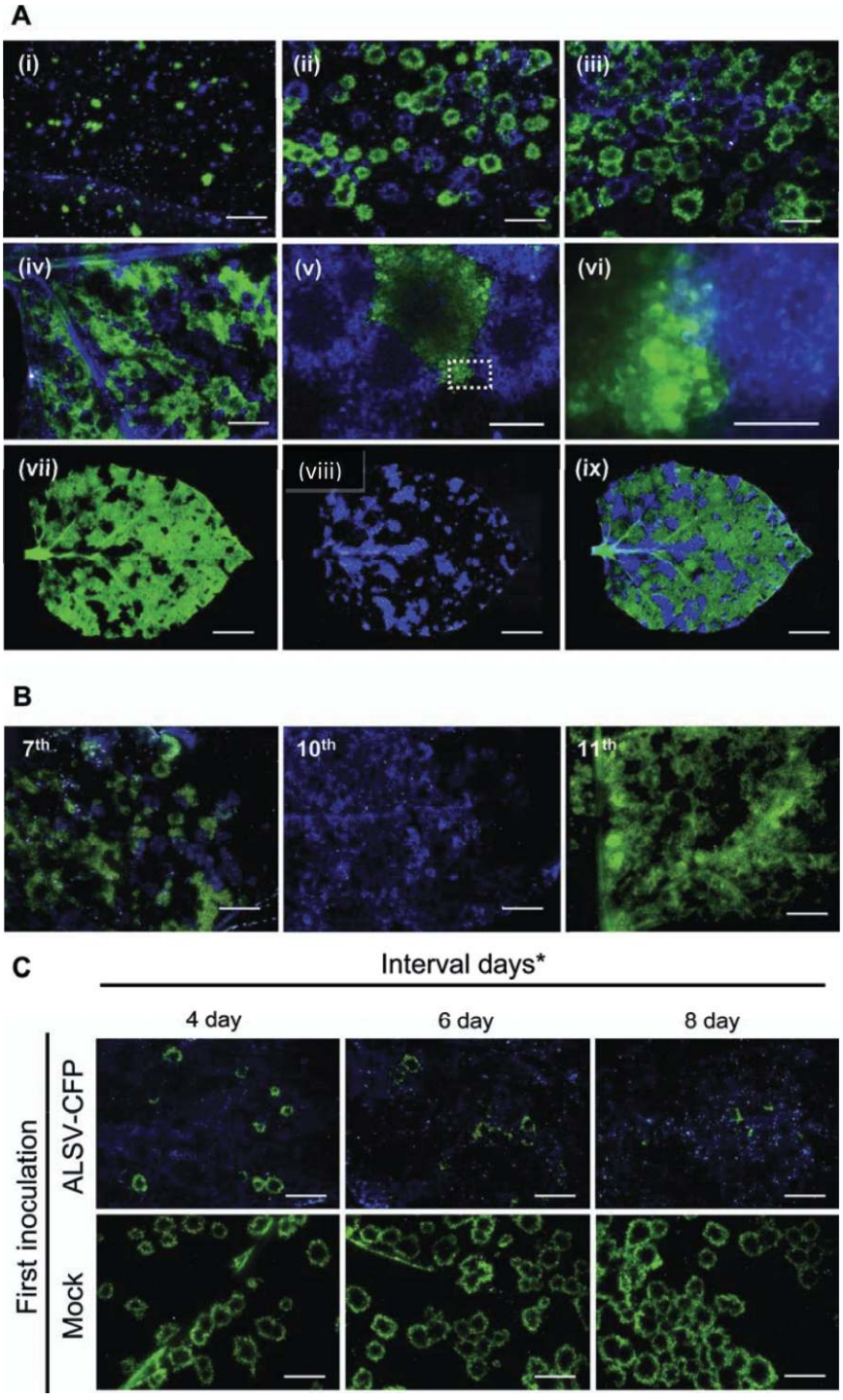

Fig. 3 Analysis of the distribution of identical, but differently labelled, virus populations in co-inoculated plants. (A) The fluorescence of cyan fluorescent protein (CFP) or yellow fluorescent protein (YFP) on inoculated leaves at 3 days post-inoculation (dpi) (i), 5 dpi (ii), 6 dpi (iii) and upper uninoculated leaves at 11 dpi (iv, v and vi) of *Chenopodium quinoa* plants infected with *Apple latent spherical virus* expressing CFP (ALSV-CFP) and YFP (ALSV-YFP). A border area between populations of ALSV-YFP and ALSV-CFP in (v) is shown in (vi). The fluorescence of YFP (vii) and CFP (viii) on an upper leaf of *Nicotiana benthamiana* mixed infected with *Bean yellow mosaic virus* expressing CFP (BYMV-CFP) and BYMV-YFP. (ix) is a merged image of (vii) and (viii). (B) Distributions of ALSV-CFP and ALSV-YFP among different upper leaves of a *C. quinoa* plant at 10 dpi. A *C. quinoa* plant was inoculated with ALSV-CFP to the third and fifth true leaves and ALSV-YFP to the fourth and sixth leaves. Both YFP and CFP fluorescence signals were detected in a seventh leaf (left panel). CFP and YFP were separately distributed on the 10th (centre panel) and on the 11th leaves (right panel), respectively. (C) YFP spots at 7 days after ALSV-YFP inoculation on *C. quinoa* leaves that were first inoculated with ALSV-CFP or buffer only (Mock), and then secondly inoculated with ALSV-YFP at 4, 6 or 8 days after the first inoculation. Bars: A, 2 mm in (i)–(iv), 500 μm in (v), 200 μm in (vi) and 5 mm in (vii)–(ix); B and C, 2 mm. Figure and text taken from Takahashi *et al*. (2007) with kind permission of the copyright owner The American Phytopathological Society.

replicate in a cell (González-Jara *et al*., 2009; Gutiérrez *et al*., 2010; Miyashita and Kishino, 2010). As pointed out by Elena *et al*. (2011), spatial separation reduces the opportunities for competition between viral genetic variants, thus restricting the possibilities to eliminate unfit variants and, consequently, to increase the overall population fitness. Moreover, the spatial plant structure hampers the occurrence of beneficial mutations within the viral metapopulation, irrespective of their advantageous effect within a particular spatial level. Beneficial mutations in cells that are confined by other infected cells remain unusable, as they will be unable to spread spatially and will not contribute to selection. Furthermore, spatial distribution and mutual exclusion can strongly reduce the opportunity for recombination, and thus for the generation of genetic variation (Elena *et al*., 2011).

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FINAL REMARKS

Viruses are the fastest evolving entities existing on the Earth. Virus–host co-evolution is a continuous process involving both the host immune system and viral escape mechanisms, and is considered to be an important factor in the maintenance of genetic variation in resistance to this group of pathogens. One of the most intriguing aspects of virus–host co-evolution is the variety of pathways of interactions between the two partners. Especially important aspects are the outcomes of multiple infections in terms of the final fitness exhibited by viral populations selected by competitive interactions. When considered with regard to virus evolution, synergistic interactions between related viruses invading the same cells may result in recombination or pseudorecombination events that facilitate the emergence of novel virus variants, showing higher fitness than the parental viruses (e.g. García-Arenal *et al*., 2003; Malpica *et al*., 2006; Méndez-Lozano *et al*., 2003; Miralles *et al*., 2001; and references cited therein), thereby shaping virus populations. In addition, Elena (2011) indicated that, assuming that sequence similarity may still be significant between two members of the same family, double infection of plant cells constitutes provocative conditions that may yield interspecific recombination or pseudorecombination (also called reassortment), and thus the generation of new pathogen species. However, it has been repeatedly demonstrated that closely related viruses tend to be spatially separated in plants (Dietrich and Maiss, 2003; Takahashi *et al*., 2007; Takeshita *et al*., 2004). This spatial structuring of viral genotypes during infection, associated with relatively low MOI values, may be a significant constraint on the occurrence of recombination (Elena *et al*., 2011). The rate of recombination is an important factor determining the level of genetic diversity within the virus population. Consequently, a low MOI reduces genetic diversity, whereas a high MOI favours it. As speculated by González-Jara *et al*. (2009), high diversity is advantageous to viruses at the beginning of host colonization, but, later on, limiting co-infection would be an advantage for the fittest genomes.

Whether or not spatial separation of related viruses in double infection occurs commonly in nature and what mechanisms are behind this phenomenon remain largely unknown (Fabre *et al*., 2009; Roossinck, 2005). Nevertheless, the emergence of infectious recombinants or pseudorecombinants following natural mixed infections with begomoviruses (family *Geminiviridae*) has frequently been reported (Méndez-Lozano *et al*., 2003; Pita *et al*., 2001; and references cited therein). More recently, the occurrence of a more virulent pseudorecombinant between two begomoviruses has been reported by Chakraborty *et al*. (2008). It should be emphasized that a novel viral variant often shows altered virus–host and/or virus–vector interactions that determine the virus host range, rate of transmission, or both.

The biological and epidemiological consequences of both facilitative and antagonistic virus–virus interactions seem to be merely predictable, or just unpredictable. In this review, it has been shown that synergistic interactions may occur between unrelated or related plant viruses, which means that the relatedness between viruses is not a strong barrier for the phenomenon to occur. However, the spatial distribution of closely related viruses within the host plant is the manifestation of antagonism rather than synergism between the viruses belonging to the same species or genus. So far, the mechanisms for facilitative or antagonistic interactions between viruses in multiple infections are either only partly recognized, or remain hypothetical, and thus require further detailed studies. This review was not intended to provide a comprehensive overview of all the literature data related to the problem of virus–virus interactions in mixed infections of plants, but rather to exemplify the phenomena and their outcomes that seem to be the most interesting and most important from both the virological and agricultural points of view.

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