FOCUSED REVIEW

Inter-virus relationships in mixed infections and virus-drought relationships in plants: a quantitative review

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SUMMARY

Inter-virus relationships in mixed infections and virus-drought relationships are important in agriculture and natural vegetation. In this quantitative review, we sampled published factorial experiments to probe for relationships against the null hypothesis of additivity. Our sample captured antagonistic, additive and synergistic inter-virus relationships in double infections. Virus-drought relationships in our sample were additive or antagonistic, reinforcing the notion that viruses have neutral or positive effects on droughted plants, or that drought enhances plant tolerance to viruses. Both inter-virus and virus-drought relationships vary with virus species, host plant to the level of cultivar or accession, timing of infection, plant age and trait and growing conditions. The trait-dependence of these relationships has implications for resource allocation in plants. Owing to lagging theories, more experimental research in these fields is bound to return phenomenological outcomes. Theoretical work can advance in two complementary directions. First, the effective theory models the behaviour of the system without specifying all the underlying causes that lead to system state change. Second, mechanistic theory based on a nuanced view of the plant phenotype that explicitly considers downward causation; the influence of the plant phenotype on inter-virus relations and vice versa; the impact of timing, intensity and duration of drought interacting with viruses to modulate the plant phenotype; both the soil (moisture) and atmospheric (vapour pressure deficit) aspects of drought. Theories should scale in time, from short term to full growing season, and in levels of organisation up to the relevant traits: crop yield in agriculture and fitness in nature.

Keywords: water, antagonism, synergy, phenotype, allocation, agriculture, ecology.

INTRODUCTION

Multiple stresses are often non-additive and interactions are a fundamental cause of ecosystem complexity (Fereres et al., 1996; Mooney et al., 1991; Sih et al., 2004; Wootton, 2002). The decoupling of trophic webs is a conspicuous, ecologically significant and agronomically important effect of global change (Edwards & Richardson, 2004; Han et al., 2019; Kankaanpää et al., 2020). Worldwide, many cropping areas are becoming not only hotter but also drier (IPCC, 2022). In the absence of technological adaptation, higher temperature could reduce the yield of grain crops by shortening the critical period of grain set in mid-latitudes, compounding the effect of agronomically relevant drought (Passioura, 2006a), and by increasing pest damage (Canto et al., 2009; Deutsch et al., 2018; Slafer et al., 2023; Trebicki et al., 2017). Warming could increase the population growth rate of many globally important insect pests in mid-latitudes, and projected rates of increase in insect damage are 10–25% per °C (Deutsch et al., 2018). In particular, Hemiptera species that damage plants directly by feeding and indirectly as vectors of plant viruses are expected to expand their geographical distribution and cause more damage to crops and wild vegetation in future climates (Bebber et al., 2013; Canto et al., 2009; Deutsch et al., 2018; Jeger, 2022; Trebicki, 2020; Trebicki et al., 2017).

On evolutionary time scales, conflicts between host and parasite are resolved into multiple evolutionary stable
regimes that span from highly aggressive parasites, such as lytic viruses that kill the host and move to the next one, to cooperative elements, such as plastids, which provide beneficial functionalities to the host (Koonin et al., 2021). Inter-virus relationships are relevant because multiple infections are common in crops and natural vegetation, and have implications for viral evolution and ecology including the emergence of new variants, pathogenesis and control practices (Avilla, Collar, Duque, & Fereres, 1997; Jeger, 2020; Juarez et al., 2013; Koonin et al., 2021; Mascia & Gallitelli, 2016; McLeish et al., 2019; Piccinni & Rush, 2000; Syller, 2012; Vijaykrishna et al., 2015). Inter-virus relationships can be investigated with a focus on the virus, the plant or the insect vector. An insect-centred perspective is outside the scope of this paper.

From a virus-centred perspective, synergistic relationships increase and antagonistic relationships decrease, replication, accumulation or concentration, within host movement and transmission rate of one or both interacting viruses (Barker, 1989; Mascia & Gallitelli, 2016; Mendez-Lozano et al., 2003; Syller, 2012). Relationships can be asymmetric; for example, synergistic for one of the viruses and antagonistic or additive for the other (Mendez-Lozano et al., 2003; Singhal et al., 2021). In mixed infections, a given virus might provide a movement function to a transport-deficient virus, increasing symptom severity and increasing the concentration of the dependent virus in the host plant (Takeshita & Takanami, 2000). Some non-circulative viruses may interfere with each other for transmission by aphids, for example, cucumber mosaic virus (CMV) and zucchini yellow mosaic virus (ZYMV), but others do not, for example, cauliflower mosaic virus (CaMV) and turnip mosaic virus (TuMV) (Jimenez, 2019). This could be partially related to competition between viruses in the aphid’s acrostyle, located in the common duct where the salivary and food canal fuse together (Uzest et al., 2007), but competition between viruses within the host plant is also possible.

From a plant-centred perspective, where the focus is the plant phenotype, inter-virus relationships can also be antagonistic, additive or synergistic. For example, the combination of CMV and watermelon mosaic virus 2 (WMV-2) was antagonistic for the yield of field-grown melon under the experimental conditions of Alonso-Prados et al. (1997). The combined infection of cowpea with CMV and blackeye cowpea mosaic virus (BICMV) reduced the yield of glasshouse-grown cowpea synergistically in the study of Pio-Ribeiro et al. (1978). Importantly, plant (e.g. disease symptoms) and virus (e.g. accumulation) responses to multiple infections are not necessarily coupled (Berges et al., 2021; Gonzalez-Jara et al., 2004; Mascia & Gallitelli, 2016; Wang et al., 2002).

Empirical evidence supports an interaction whereby virus infection reduces the survival, growth and reproduction of well-watered plants, with neutral or positive effects under drought stress (Aguilar et al., 2017; Berges et al., 2020; Davis et al., 2015; Xu et al., 2008). Inoculation with CaMV was neutral or enhanced the survival rate of droughted Arabidopsis thaliana seedlings in comparison to well-watered controls, for example, from 10% in well-watered plants to 55% under drought for Ini-0, and from 30% to 95% for Cdm-0, whereas the survival rate of inoculated seedlings was 100% for some accessions regardless of water regime (Berges et al., 2020). Inoculation with TuMV that underwent five-passage evolution in droughted A. thaliana altered the expression of plant genes related to the circadian clock, phytohormone synthesis and growth signalling pathway, and improved seedling survival in three out of four accessions in comparison to plants inoculated with TuMV evolved in well-watered plants (Curin et al., 2021). This led to the conclusion that droughted plants become more tolerant to virus infection than well-watered plants (Curin et al., 2021).

Inter-virus relationships in mixed infections and virus-drought relationships have been reviewed with a focus on diagnostics and control, impacts on host plant, molecular mechanisms and evolutionary, ecological, epidemiological and agronomic implications (Alazem & Lin, 2015; Prasad et al., 2022; Singhal et al., 2021; Syller, 2012; Szczepaniec & Finke, 2019; van Munster, 2020). All these reviews are narrative. Here we quantify the nature of both inter-virus relationships in mixed infections and virus-drought relationships. We sample published studies to quantitatively re-assess relationships against the null hypothesis of additive effects, and explore departures from additivity associated with virus species, host plant genotype, timing of infection and plant age, plant trait and growing conditions. Agronomic and ecological implications of these relationships are outlined.

**QUANTIFICATION OF INTER-VIRUS RELATIONSHIPS AND VIRUS-DROUGHT RELATIONSHIPS**

We emphasise ‘relationships’ rather than ‘interactions’, the term favoured in the literature (Mascia & Gallitelli, 2016; Prasad et al., 2022; Syller, 2012), because interactions might bias interpretations against additivity. We inspected the literature cited in the reviews above and searched papers in Web of Science using combinations of terms including ‘virus’ and ‘mixed infection’, ‘virus’ and ‘drought’, ‘virus’ and ‘water’. We did not attempt to apply the rules of meta-analysis for data search and did not focus on effect size (Koricheva & Gurevitch, 2014; Philibert et al., 2012). Instead, our search targeted factorial experiments reporting the effects of multiple virus infection or combined virus and water regime treatments on traits of the plant (e.g. photosynthetic rate, hormone concentration, gene expression) and virus (e.g. abundance, transmission). Studies where relationships between viruses or between...
INTER-VIRUS RELATIONSHIPS IN DOUBLE INFECTIONS

To investigate inter-virus relationships in double infections we targeted papers with factorial experiments including at least the four combinations of virus treatments, that is, mock-inoculated control, inoculated with virus 1, inoculated with virus 2 and inoculated with both viruses. To investigate virus-drought relationships we targeted factorial experiments including at least the four combinations of virus and water regime, that is, mock-inoculated, well-watered control; mock-inoculated, water stressed; virus inoculated, well-watered; virus inoculated, virus stressed.

From a plant-centred perspective, we quantified inter-virus relationships in double-infected plants with the method of Grace (1995) modified by Sadras (1997). The effect of virus 1 (Δv₁) and virus 2 (Δv₂) on plant trait T was calculated as the difference with the mock-inoculated control:

\[
\Delta v_1 = T (\text{control}) - T (\text{virus 1}) \tag{1a}
\]

\[
\Delta v_2 = T (\text{control}) - T (\text{virus 2}) \tag{1b}
\]

The actual effect of both viruses in the double infection was calculated as:

\[
\Delta v_1 + \Delta v_2 = T (\text{control}) - T (\text{virus 1 + 2}) \tag{2}
\]

The expected effect assuming additivity is:

\[\text{Additive (virus 1 + virus 2) = \Delta v_1 + \Delta v_2} \tag{3}\]

The actual-to-additive ratio, Equation (2)/Equation (3), indicates the type of inter-virus relationship:

- Ratio >1, synergistic
- Ratio = 1, additive
- Ratio <1, antagonistic

From a virus-centred perspective, we used the approach of Barker (1989) and Tatineni et al. (2010) to calculate the actual-to-additive ratio. For example, if abundance of virus 1 in single inoculated plants is 100, and its abundance under co-infection with virus 2 is 50, 100 or 200, the ratio is 0.5 indicating antagonism, 1 indicating additivity and 2 indicating synergy, respectively. This assumes that virus abundance in the mock-inoculated control is negligible.

Similar calculations were used to assess virus-drought relationships in factorial experiments where plants were grown under contrasting water regimes. The effect of virus (Δv) and water stress (Δw) on plant trait T was calculated as the difference with the mock-inoculated, well-watered control:

\[
\Delta v = T (\text{control}) - T (\text{virus}) \tag{4a}
\]

\[
\Delta w = T (\text{control}) - T (\text{water stress}) \tag{4b}
\]

The actual effect of virus and water stress was calculated as:

\[
\Delta v + \Delta w = T (\text{control}) - T (\text{virus + water stress}) \tag{5}
\]

The expected effect assuming additivity is:

\[\text{Additive (virus + water) = \Delta v + \Delta w} \tag{6}\]

The actual-to-additive ratio was calculated as Equation (5)/Equation (6).

We analysed statistically the type of relationship against the null hypothesis of additivity with two complementary approaches. First, where standard errors (SE) or standard deviations (SD) of means were reported, we propagated errors (Table S1) and calculated the 95% confidence interval for the actual-to-additive ratio. Second, we considered the statistical analysis reported in the original paper including p for interactions from ANOVA and post hoc mean comparisons.
Inter-virus and virus-drought relationships

Inter-virus relationships in mixed infections are host-dependent at the level of species, and cultivars or accessions (Anderson et al., 1999; Mendez-Lozano et al., 2003; Murphy & Kyle, 1995; Tatineni et al., 2010). For example, the relationship between pepper huasteco virus (PHV) and pepper golden mosaic virus (PepGMV), quantified with plant symptoms on a scale from 1 (small yellow spots visible only against a strong light) to 10 (severe plant stunting with curling and rugosity), was antagonistic in pepper and synergistic in tobacco and Nicotiana benthamiana (Mendez-Lozano et al., 2003). The relationship between pepper mottle virus (PepMoV) and CMV, quantified with virus antigen on leaves, was additive in glasshouse-grown bell pepper NuMex R Naky and asymmetrically synergistic in Avelar, where the amount of CMV antigen did not depart from additivity in double infection and increased 4.3-fold for PepMoV in the presence of CMV compared to singly inoculated plants (Figure 1c). In glasshouse-grown cowpea, the actual-to-additive ratio for foliar fresh weight in response to co-infection with CMV and BICMV varied with cultivar from antagonistic in PPH-BVR to additive in Excel-68 to synergistic in Coronet and Excel-71 (Figure 1d). Lack of standard errors in the original precluded calculation of confidence interval, but statistical tests for mean comparisons (Table 1 in Anderson et al., 1996) and virus accumulation (Figure 3b in Anderson et al., 1996) support the cultivar-dependent variation in inter-virus relationship. In PPH-BVR, accumulation of CMV was similarly low in singly and doubly inoculated plants and accumulation of BICMV was similarly low in both singly and doubly inoculated plants; virus co-infection in this variety with extreme resistance to BICMV neither broke the resistance to BICMV nor resulted in any interaction with CMV accumulation or disease (Palukaitis & García-Arenal, 2003). The apparent additive effect in Excel-68 was consistent with an enhanced accumulation of CMV in the presence of BICMV that was cancelled by a reduced accumulation of BICMV in the presence of CMV. The apparent synergy in Coronet and Excel-71 was primarily associated with a marked enhancement of CMV in the presence of BICMV (Anderson et al., 1996).

Inter-virus relationships are trait-dependent (Figure 2). In field-grown sugar beet (Beta vulgaris) the relationship delayed inoculation (Figure 1a), indicating that mature plant resistance did not hold in doubly infected plants.

Inter-virus relationships, quantified as the time for a virus to reach a leaf 3 nodes above the inoculated leaf, varied with the time lag between the inoculation of individual viruses, as illustrated in glasshouse-grown tobacco seedlings co-inoculated with PVY and two strains of potato virus X (PVX) (Goodman & Ross, 1974). Quantification of viral DNA in leaves of greenhouse-grown melon (Cucumis melo) showed that the relationship between squash leaf curl virus (SLCV) and watermelon chlorotic stunt virus (WmCSV) shifted from additive to synergistic between 6 and 24 days post inoculation (dpi) for SLCV, with a linear increase in the actual-to-additive ratio at 0.13 day−1 (Figure 1b). The ratio varied non-linearly with time for WmCSV, shifting from additive at 6 dpi to transiently synergistic at 12 dpi, returning to additive afterwards (Figure 1b). Similarly, the concentration of potato leafroll luteovirus (PLRV) in Nicotiana clevelandii seedlings in response to co-inoculation with carrot mottle virus (CMoTvo) was additive at 8 dpi, transiently synergistic at 12 days (actual-to-additive ratio > 5.9), returning to additive at 18 dpi (Barker, 1989). In comparison, the concentration of PLRV co-inoculated with pea early-browning tobavirus strain SHE (PEBV-SHE) shifted from additive at 8 dpi to synergistic at 12 dpi, and the synergy persisted at 18 dpi (actual-to-additive ratio > 7.1) (Barker, 1989).

Inter-virus relationships in mixed infections vary with time of inoculation, plant age and cultivar. Figure 1. Relationships between viruses in mixed infections vary with time of inoculation, plant age and cultivar.

(a) The relation between cucumber mosaic virus (CMV) and potato virus Y (PVY) in bell pepper, quantified for fruit yield (g plant−1) in two growing seasons, varied with time of inoculation.

(b) The relation between squash leaf curl virus (SLCV) and watermelon chlorotic stunt virus (WmCSV) in melon, quantified for viral DNA accumulation, varied with plant ontogeny. (c) Relation between pepper mottle virus (PepMoV) and cucumber mosaic virus (CMV), quantified for virus antigen on Capsicum annum leaves, varied between cultivars NuMex R Naky and Alvar. (d) The relation between CMV and blackeye cowpea mosaic virus (BICMV), quantified for foliar fresh weight (g plant−1), varied with cowpea cultivar. In all graphs, the vertical line (ratio = 1) is the boundary between antagonism and synergy, and error bars are 95% confidence intervals; lack of standard errors in the original publication precluded the calculation of confidence intervals in (d). Calculated from data in (a) Avilla, Collar, Duque, and Ferreres (1997), (b) spring experiment in Sufrin-Ringwald and Lapidot (2011), (c) Murphy and Kyle (1995), (d) Anderson et al. (1996).
between beet soilborne mosaic virus (BSBMV) and beet necrotic yellow vein virus (BNYYV) was antagonistic, with an actual-to-additive ratio for a yield of 0.3 (Figure 2a); this is supported statistically by a post hoc test showing the yield of doubly inoculated crops was between the yields of single-virus treatments (inset Figure 2a). The actual-to-additive ratio for disease symptoms, in a scale from 0 (healthy plant) to 4 (small, hairy and stunted or rotted beet-root), was 1.7 in 1996 and 1.4 in 1997 indicating a synergistic interaction from ANOVA in the original study is included for comparison. Calculated from data in Sacco Botto et al. (2023)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Ratio</th>
<th>Relationshipa</th>
<th>P for interactionc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem water</td>
<td>0.55 ± 0.20</td>
<td>Antagonistic</td>
<td>0.0066</td>
</tr>
<tr>
<td>Plant height</td>
<td>0.82 ± 0.12</td>
<td>Antagonistic</td>
<td>0.96</td>
</tr>
<tr>
<td>Fresh weight</td>
<td>0.91 ± 0.67</td>
<td>Additive</td>
<td>0.12</td>
</tr>
<tr>
<td>Root length</td>
<td>1.55 ± 0.84</td>
<td>Additive</td>
<td>0.0005</td>
</tr>
<tr>
<td>ABA</td>
<td>3.33 ± 4.04</td>
<td>Additive</td>
<td>0.89</td>
</tr>
<tr>
<td>IAAS</td>
<td>1.01 ± 0.84</td>
<td>Additive</td>
<td>0.29</td>
</tr>
<tr>
<td>SINCED1</td>
<td>2.80 ± 12.86</td>
<td>Additive</td>
<td>0.80</td>
</tr>
<tr>
<td>SICYP707A2</td>
<td>0.24 ± 0.20</td>
<td>Antagonetic</td>
<td>0.0001</td>
</tr>
<tr>
<td>SITAS14</td>
<td>1.76 ± 1.04</td>
<td>Additive</td>
<td>0.14</td>
</tr>
<tr>
<td>SIARF8</td>
<td>1.00 ± 0.50</td>
<td>Additive</td>
<td>0.11</td>
</tr>
</tbody>
</table>

*Relationship based on actual-to-additive ratio.
*Not calculated because SA was below detection in mock-inoculated, well-watered control.
*P for interaction from ANOVA included two virus treatments and three water regimes: well-watered control, severe water stress of shorter duration in mock-inoculated than in TYLCSV-inoculated plants and a re-watering treatment.
*Not reported in the original paper.

The actual-to-additive ratio for the relationship between wheat streak mosaic virus (WSMV) and triticum mosaic virus (TriMV), quantified with virus concentration measured with RT-PCR, varied with virus species, wheat (Triticum aestivum) cultivar, growing condition and time after inoculation (Figure 3). Out of the 24 cases resulting from the combination of these factors, inter-virus relationships were synergistic in 12, antagonistic in 7 and additive in 5 (Figure 3c). There were intricate virus-dependent shifts in the actual-to-additive ratio with time, that were cultivar and growing condition dependent. For example, in cv. Arapahoe the relationship was synergistic for both viruses at both 14 and 28 dpi in chambers but shifted from synergistic at 14 dpi to antagonistic for WSMV and additive for TriMV at 28 dpi (Figure 3). For cv. Mace, the relationship between viruses evaluated with MSMV at 14 dpi was additive in the chamber and antagonistic in the glasshouse and shifted to antagonistic at 28 dpi in the chamber and to synergistic in the glasshouse. The glasshouse was hotter than the chamber, but the effect of temperature was confined with the total amount and diurnal pattern of radiation, hence our focus on growing conditions, rather than temperature in the original study (Tatineni et al., 2010).

**Agronomic and ecological implications**

Our sample of studies returned 18% antagonistic, 48% additive and 33% synergistic inter-virus relationships in double infections (Figures 1-3). The trait-dependence of inter-virus relationships is agronomically and ecologically important because it implies mixed infections can change the pattern of resource allocation in plants. The additive relationship between CMV and BICMV for vegetative growth and their synergistic relationship for yield represents a reduction in reproductive allocation (Figure 2b). Both reproductive allocation and allocation among vegetative organs influence plant fitness in nature (Hiltbrunner et al., 2021; Körner, 1981; Weiner, 2004). With few exceptions, increased reproductive allocation has been the main driver of genetic gain in the yield of most grain crops over the last century (Fischer et al., 2014; Liu et al., 2021; Slauer, 1994). Tolerance to CMV of Arabidopsis varied among allocation types whereby long-lived genotypes with low
reproductive allocation were more tolerant than short-lived genotypes with high seed-to-biomass ratio (Hily et al., 2016). Tolerance in the former group partially associated with delayed flowering (Hily et al., 2016).

Another implication of the trait-dependence of inter-virus relationships is that conclusions based on a single trait, Figure 2. Relationships between viruses in mixed infections are trait-dependent.

(a) The relationship between beet soilborne mosaic virus (BSBMV) and beet necrotic yellow vein virus (BNYVV) was antagonistic for yield (ratio < 1) and synergistic for disease symptoms (ratio > 1). Inset shows that the yield of the doubly infected crop was between the yield of crops infected with each virus individually; letters indicate different means from Duncan’s test ($P < 0.05$) reported in the original study.

(b) The relationship between cucumber mosaic virus (CMV) and blackeye cowpea mosaic virus (BICMV) shifted from near additive for vegetative traits to synergistic for yield in cowpea. Traits are from top to bottom fruit yield (g plant$^{-1}$), leaf number (plant$^{-1}$), leaf weight (g plant$^{-1}$), plant height (cm), stem weight (g plant$^{-1}$) and root weight (g plant$^{-1}$).

(c) The relationship between CMV and watermelon mosaic 2 potyvirus (WMV-2) was antagonistic for fruit number (plant$^{-1}$) and fruit yield (kg plant$^{-1}$) and additive for three fruit morphological traits in melon including polar diameter ($D_{\text{polar}}$), equatorial diameter ($D_{\text{equatorial}}$) and flesh thickness (all in cm); error bars are 95% CI.

(d) The relationship between sweet potato mild mottle virus (SPMMV) and CMV, C-6 virus (C-6) and sweet potato chlorotic stunt virus (SPCSV) varied with crop trait in field-grown sweet potato. Traits are total storage root number (TSRno, plot$^{-1}$), marketable storage root weight (MSRwt, kg plot$^{-1}$), marketable storage root number (MSRno, plot$^{-1}$), ground cover at 50 days after planting (GC, cm$^2$ plant$^{-1}$), fresh leaf weight at 160 days after planting (leaf wt, kg plot$^{-1}$) and total storage root weight (TSRwt, kg plot$^{-1}$). In all figures, the vertical line (ratio = 1) is the boundary between antagonism and synergy. Calculated from data in (a) Piccinni and Rush (2000), (b) Pio-Ribeiro et al. (1978), (c) Alonso-Prados et al. (1997), (d) Untiveros et al. (2007).
such as virus accumulation, disease symptoms or plant yield are incomplete (e.g. Gonzalez-Jara et al., 2004; Pio-Ribeiro et al., 1978; Pruss et al., 1997). Furthermore, the nature of inter-virus relationships varies with time of infection, plant age, host genotype and growing conditions; caution is needed in our attempt to generalise inter-virus relationships. We emphasise the need for both rigorous statistics to probe for interactions and to make explicit the context of experimental findings, which are lacking in many studies. For example, the relationship between WSMV and TriMV for the abundance of TriMV was additive for 14–28 days old, glasshouse-grown Mace wheat (Figure 3); this is cumbersome but helps to avoid undue generalisations.

The variation in inter-virus relationships with time of infection and plant age in doubly infected plants has agronomic and ecological consequences (Figure 1). Syller (2012) distinguishes co-infection, where two or more viruses invade the host more or less simultaneously, and super-infection when the lag between infections is longer. In natural conditions, the early stages of infection combine a high availability of host individuals in a crop stand and low viral density, leading to a high likelihood of single-virus infection. With time, both the number of infected plants and viral load increase, thereby increasing the probability of mixed infections (Syller, 2012). In this context, using virus-free propagation material and early control of insect vectors would reduce the chances of multiple virus infections (Syller, 2012). Practices to reduce the risk of multiple virus infections include insect-proof or UV-absorbing nets, plant barriers, reflective mulches and other optical barriers and floating row covers, which are more effective when implemented early in the cropping season (Antignus, 2012; Avilla, Collar, Duque, Perez, & Fereres, 1997).

VIRUS-DROUGHT RELATIONSHIPS VARY WITH VIRUS SPECIES; HOST SPECIES, ACCESSION OR CULTIVAR; PLANT TRAIT; ENDOPHYTE COLONISATION; PLANT GROWING CONDITIONS

Actual-to-additive ratios for virus-drought relationships

Experimental evidence and incipient theory partially support an antagonistic relationship between virus and drought stress, whereby the reduction in plant growth and yield in response to the combination of both stresses is less than expected from the individual effect of each stress. Figure 4a illustrates the antagonistic effect between barley yellow dwarf virus (BYDV) and drought stress on the growth and yield of glasshouse-grown wheat. Shoot dry matter was reduced from 7.5 g plant\(^{-1}\) in mock-inoculated, well-watered controls to 2.3 g plant\(^{-1}\) in mock-inoculated, water-stressed plants (\(\Delta w = 5.2 \) g plant\(^{-1}\)) and to 5.6 g plant\(^{-1}\) in BYDV-inoculated, water-stressed plants (\(\Delta v = 1.9 \) g plant\(^{-1}\)), hence the expected additive effect \(\sim 7.1 \) g plant\(^{-1}\). In comparison, the actual reduction in shoot dry matter in droughted, BYDV-infected plants was 5.9 g plant\(^{-1}\), returning an actual-to-additive ratio \(\sim 0.7\) (Figure 4a). The reduction in seed number and seed yield was also \(\sim 0.7\) of the expected additive effect (Figure 4a). This kind of response has been interpreted in terms of enhanced drought adaptation in virus-infected plants in the study illustrated in Figure 4a (Davis et al., 2015) and in

![Figure 3](image-url)
many others (Berges et al., 2021; Mishra et al., 2022; Moreno et al., 2022; Xu et al., 2008). The equally plausible, alternative interpretation, namely drought enhances tolerance to virus, is less common (Curin et al., 2021). Experiments, where the timing of virus infection varies relative to the timing of water stress, can be useful to untangle the causal link. Enhanced drought tolerance in virus-infected plants has been analysed extensively; it has been attributed to increased levels of antioxidants and osmoprotectants partially mediated by increased protein hydroxylation, interference with crosstalk between abscisic acid (ABA) and salicylic acid (SA) and modulation of the relationship between plant and insect vector (Mishra et al., 2022; Prasad et al., 2022; Szczepaniec & Finke, 2019; van Munster, 2020). Shifts in gene expression and common metabolic pathways between plant defence and response to drought stress are insightful but fall short in relevance in the absence of scaling. In the context of genomic prediction of functional processes of health and disease at higher levels of physiological organisation, Noble

Figure 4. Relationships between virus and water stress vary with plant accession or cultivar, plant trait, growing condition and endophyte colonisation. (a) Relationship between barley yellow dwarf virus (BYDV) and drought quantified for shoot dry matter (g plant$^{-1}$) and seed yield (g plant$^{-1}$) in glasshouse-grown wheat. (b) Relationship between cauliflower mosaic virus (CaMV) and drought quantified for rosette dry mass (mg) in 25 A. thaliana accessions. (c) Relationship between grapevine leaf-roll-associated virus-3 (GLRaV-3) and plant water deficit for net photosynthetic rate (AN, μmol CO₂ m⁻² s⁻¹), maximum photosynthetic electron transport (Fv’max, μmol CO₂ m⁻² s⁻¹), maximum carboxylation rate (Vcmax, μmol CO₂ m⁻² s⁻¹), stomatal conductance (gs, mmol H₂O m⁻² s⁻¹), leaf transpiration rate (E, mmol H₂O m⁻² s⁻¹), plant hydraulic conductivity (Kh, mmol MPa⁻¹ m⁻¹), leaf thickness (T, μm), leaf mass per area (M₅, g m⁻²), mesophyll porosity (λₚ, %). Measurements in two grapevine cultivars, Malvasia de Banyalbufar and Giró Ros, over several growing seasons depending on trait. (d, e) Relationship between cauliflower mosaic virus (CaMV) and drought quantified for the height of flowering branch (cm) in 20 A. thaliana accessions. (f) Relationship between tailflower mild mottle virus (YTMMV) and water deficit in Nicotiana benthamiana. Traits are shoot dry weight (mg plant$^{-1}$), root dry weight (mg plant$^{-1}$) and ROS sensitivity, measured as the photobleaching of leaf discs with herbicide paraquat. In addition to the virus and water regime, three endophyte treatments were included: plants inoculated with ascomycete endophytic fungal strain E-162, strain E-284 and control with no endophyte. (g) Relationship between tomato yellow curl virus (TYLCV) and water deficit in tomato seedlings. Traits are shoot dry weight biomass (g plant$^{-1}$) and root dry weight (g plant$^{-1}$) in two TYLCV-sensitive lines (black symbols) and a TYLCV-resistant line (blue symbol). In all figures, the vertical line (ratio = 1) is the boundary between antagonism and synergy; error bars are 95% CI. Calculated from data in (a) Davis et al. (2015); (b, c) Berges et al. (2020); (d, e) El Aou-Uaoud et al., 2016, El Aou-Uaoud et al., 2017; (f) Dastogeer et al. (2018); (g) Mishra et al. (2022) and Shteinberg et al. (2021).
and Noble (2023) concluded ‘high level insights predict lower level properties, but the predictive power only rarely goes the other way’; below we re-analyse the study by Sacco Bottto et al. (2023) to further emphasise scaling issues.

Moreover, the antagonistic relationship between virus and drought stress is far from general; here we show how it varies with host accession or cultivar, plant trait, endophyte colonisation and plant growing conditions (Figure 4). In a collection of 25 A. thaliana accessions from the Iberian Peninsula, the relationship between cauliflower mosaic virus CaMV and drought stress, quantified with seedling biomass, was antagonistic for 12 and additive for 13 accessions, highlighting the genotype-dependence of the relationship between virus and drought (Figure 4b). The reduction in shoot dry matter associated with CaMV was unrelated to virus accumulation, despite a large variation among accessions, that is, 4.8-fold under drought and 20.9-fold in well-watered plants (Figure 4 in Berges et al., 2021). In comparison, the relationship between CaMV and drought stress, quantified with height of flowering branch, was antagonistic for all 20 accessions tested, with a variation in actual-to-additive ratio from 0.35 to 0.73 (Figure 4c). The lack of additive relations for height of flowering branch (Figure 4c) in contrast to ~50% of the accessions returning additive relations for seedling biomass (Figure 4b) highlights the trait-dependence of the relationship between virus and drought stress.

The genotype and trait dependence of the relationship between virus and drought stress also holds for long-lived perennials. In grapevine plants grown from cuttings in pots outdoors, the relationship between grapevine leaf-roll-associated virus-3 (GLRaV-3) and drought stress was antagonistic for net assimilation rate (2013), stomatal conductance (2013, 2014), leaf transpiration (2014) and plant hydraulic conductivity (2014) in cultivar Malvasia de Banyalbufar, and additive for all gas exchange and water conductivity traits in Giró Ros (Figure 4d,e). Of three morphological traits measured in 2013, the relationship was antagonistic for leaf thickness in both cultivars and for leaf mass per unit area in Malvasia, and additive for mesophyll porosity in both cultivars (Figure 4d,e). A negative actual-to-additive ratio indicates the reversal of a plant phenotypic response to the combined stresses. For example, leaf thickness of Malvasia increased from 150 μm in well-watered, mock-inoculated control to 185 μm in well-watered, inoculated plants (Δv = –35 μm, Equation 4a) and to 155 μm in water-stressed, mock-inoculated plants (Δw = –5 μm, Equation 4b); the expected additive effect was –40 μm. The actual change in leaf thickness in water-stressed, GLRaV-3 inoculated plants relative to controls was 16 μm, that is, plants under both stresses had thinner rather than thicker leaves, hence the actual-to-additive ratio of –0.40 (Figure 4d). Thus, water stress reduced the thickness of grapevine leaves infected with GLRaV-3 under these experimental conditions.

The relationship between yellow tailflower mild mottle virus (YTMMV) and drought stress in greenhouse-grown Nicotiana benthamiana varied with both trait and ascomycete endophytic fungal strain colonisation (Figure 4f). In plants with no endophyte, the relation was additive for shoot biomass and antagonistic for root biomass, and the relation was additive for both shoot and root biomass in plants inoculated with endophytes (Figure 4c). The production and scavenging of reactive oxygen species (ROS) are central to plant stress physiology (Alazem & Lin, 2015; Das & Roychoudhury, 2014; Liu & He, 2016; Pandhair & Sekhon, 2006). In the absence of YTMMV, water stress increased ROS sensitivity of plants, measured as the photobleaching of leaf discs with herbicide paraquat, by 3.8–5.4 fold in plants with endophyte and 13-fold in plants with no endophyte (Dastogeer et al., 2018). In well-watered plants, YTMMV inoculation reduced ROS sensitivity by half in plants with endophyte and had no effect on their counterparts with no endophyte (Dastogeer et al., 2018). Our quantitative approach captures the intricate three-way relationship between virus, water stress and endophyte; the relationship between virus and water stress for ROS sensitivity was additive for plants with endophyte E-162 and antagonistic for both plants with E-184 and controls with no endophyte (Figure 4f).

Infection with tomato yellow leaf curl virus (TYLCV) increased the tolerance to drought of chamber-grown, TYLCV-susceptible Ikra tomato seedlings in comparison to their mock-inoculated counterparts; this response was ascribed to reduced metabolic activity in leaves, relocation of osmolytes from shoot to root and increased metabolic activity in the root (Mishra et al., 2022). In a similar experiment combining TYLCV and drought, glasshouse-grown resistant R-GF967 tomato seedlings exposed to severe water stress during 18 days featured superior TYLCV-dependent stabilisation of osmoprotectants (soluble sugars, amino-acids) than susceptible S-967 lines, with similar profiles of stress markers (HP70, BiP, HSP90) for both lines (Shteinberg et al., 2021). However, the relationship between virus and drought stress was antagonistic for both shoot and root biomass irrespective of genotype (Figure 4g); this highlights the caution needed in scaling from molecular to whole-plant phenotype, and the trait-dependence of the relationship between virus and water deficit.

The relationship between tomato yellow leaf curl Sar dinia virus (TYLCSV) and water deficit in glasshouse-grown tomato Moneymaker was antagonistic for stem water potential, which was –0.36 MPa in well-watered, mock-inoculated controls and dropped to –1.51 MPa under water stress with no virus, but only to –0.95 MPa in water-stressed plants inoculated with TYLCSV (Sacco Bottto et al., 2023, unpublished).
et al., 2023), returning an actual-to-additive ratio of 0.55 (Table 1). Scaling to plant growth, the antagonistic effect between virus and drought stress persisted for plant height, but not for plant fresh weight and root length that showed additive effects; actual-to-additive ratios and independent ANOVA converge to support this result (Table 1). ANOVA revealed an interaction between virus and water regime for abscisic acid \( (P = 0.0009) \) but not for salicylic acid \( (P = 0.29) \) and indol-3-acetic acid (IAA, \( P = 0.89 \)) in leaves (Sacco Botto et al., 2023). The actual-to-additive ratio of 1.1 reinforces the additive of the relationship between virus and drought for IAA (Table 1). The actual-to-additive ratio \( \sim 3.3 \) for ABA has a large confidence interval but is consistent with the interaction (Table 1) and reflects the 4.0-fold increase in ABA concentration in TYLCSV-inoculated, water-stressed plants in relation to controls, compared to the 1.8-fold increase in ABA concentration in response to drought in mock-inoculated plants (Sacco Botto et al., 2023). For the expression of four hormone-related, stress-responsive genes, our actual-to-additive ratios perfectly match the conclusions from independent ANOVA (Table 1). There was no interaction between virus and drought stress for the expression of three genes: SINCED1, associated with ABA biosynthesis, ABA-responsive dehydrin SITAS14 and auxin response factor SIARF8. An antagonistic interaction between virus and water regime was detected for the expression of SICYP707A2 associated with ABA degradation. The role of ABA on plant-virus relations is poorly understood in comparison to the roles of defence metabolic pathways including jasmonic acid/ethylene, and SA, and the small interfering RNA (siRNA) antiviral machinery, both of which restrict virus propagation (Alazem & Lin, 2015). Hence, the virus-enhanced water status of the droughted plant did not scale to plant growth and cannot be linked to hormonal responses and gene expression, except for a putative correlation with the expression of SICYP707A2 and ABA metabolism; this highlights the limitations of the bottom-up, genotype-to-phenotype model (Noble, 2014). Alternative theories are available but are not mainstream, chiefly West-Eberhardt’s (2003) phenotype-first model from an evo-devo perspective and Noble’s model emphasising constraints and downward causation from a physiological perspective (Noble, 2011, 2012; Noble & Noble, 2023).

**Agronomic and ecological implications**

Our sample of studies returned 57% antagonistic and 43% additive relationships between virus and drought (Figure 4, Table 1), hence supporting the consensus that viruses have neutral or positive effects on droughted plants or that drought stress enhances virus tolerance (Aguilar et al., 2017; Davis et al., 2015; Prasad et al., 2022; Szczepaniec & Finke, 2019; van Munster, 2020; Xu et al., 2008). A single case of apparent synergy, whereby TYLCSV infection increased the ABA of droughted plants (Table 1), is consistent with this conclusion. The dominant interpretation that virus infection enhances drought adaptation partially stems from experiments where virus-infected plants are subjected to water stress weeks after virus infection.

For agronomic and ecological relevance, it is important to establish the likelihood of drought developing before or after virus infection based on models accounting for the seasonal dynamics of both viral diseases (Jeger, 2020; Leclercq-Le Quillec et al., 2000; Thackray et al., 2004) and drought patterns (Chenu, 2015; Pellegrini et al., 2022). For wheat in Australia, for example, the most severe drought has an onset at \( \sim 500 \) °C before flowering (Chenu et al., 2013), whereas the most damaging viral diseases are established earlier in the season (Jones et al., 2021). In this case, and for seed-transmitted viruses such as WSMV (Jones et al., 2021), the treatment sequence where virus infection precedes drought is relevant. Owing to the combination of climate change and adaptive cropping practices in Western Australia, wheat sown into dry soil emerges after the first 20–25 mm rainfall, leaving crops vulnerable to \( \sim 3 \)-weeks drought after emergence in 2 of 4 years and \( \sim 4-5 \)-weeks drought in one of 4 years (Chapman & Asseng, 2001); to account for this type of early-season drought affecting wheat seedlings (Khan et al., 2022), experiments should focus on how plants initially grown under water stress respond to virus infection at a later stage. The gradual development of viral diseases further highlights the need of studies where virus infection follows the establishment of the drought treatment. Owing to the secondary spread of insect vectors from plant to plant within a field, polycyclic infections are common in plant-virus epidemics. Virus spread is gradual and may take several infection cycles that last many days for non-persistent viruses (Jones, 2005). For persistently transmitted viruses, the process can be even longer because non-viruliferous insect vectors need to acquire the virus from a virus-infected source, overcome a latent period and then spread the virus to neighbouring plants (Hogenhout et al., 2008). Therefore, virus spread is often a gradual process that may take several weeks, increasing the likelihood of early-season drought affecting plants before virus infection.

With few exceptions (e.g. Alonso-Prados et al., 1997; Pio-Ribeiro et al., 1978) studies on virus-drought relationships have been conducted in controlled environments. Therefore, the agronomic and ecological implications of these studies need to be considered with caution. The conclusion of neutral or beneficial effects of viruses in water-stressed plants has to be tempered by an ambiguous definition of drought, and unrealistically experimental conditions (Annunziata et al., 2017; Aphalo & Sadas, 2021; Darwin, 1859; Passiouara, 2006b, 2007, 2012). In his compilation of data on the fertility of hybrids, Darwin (1859,
p. 247) dismisses some observations on the grounds of experimental artefacts: ‘Nearly all the plants experimented on by Gartner were potted, and apparently were kept in a chamber in his house. That these processes are often injurious to the fertility of a plant cannot be doubted’. Small pots and the imposition of sudden, severe water deficit do not allow, for example, for plant acclimation and realistic root patterns, particularly in long-lived perennials with extensive root systems (Figure 5). The metabolism of sunlight-grown plants does not match that of plants grown under artificial light regimes (Annunziata et al., 2017); field validation is essential. Moreover, where the objective is crop improvement, the pipeline from lab to field can be reversed to an effective field-to-lab approach (Thistlethwaite et al., 2020).

Individual plants in pots are the most common setting to study the relationships between virus infection and drought stress. However, traits of isolated plants do not normally scale to crop stands (Pedrò et al., 2012; Sadras, 2021), partially because gene expression is density-dependent (Bowsher et al., 2017; Geisler et al., 2012).

Of particular relevance for plant-virus relations, genes involved in the response to abiotic stress, secondary metabolism and pathogen defence were downregulated in response to high plant population density under the experimental conditions of Geisler et al. (2012).

CONCLUSION

Progress in understanding inter-virus relationships in mixed infections and virus-drought relationships requires a stronger theoretical foundation, which is lagging. With current theoretical frameworks, more experimental research in these fields is bound to return phenomenological outcomes, with results lacking contextualised interpretability. Experimental research can thus be justified in an applied context, to improve varieties and manage crops; this requires realistic settings and close engagement between scientists and practitioners. The higher-level relations involving mixed virus infection in droughted plants remain to be investigated.

Theoretical work can advance in two complementary directions. First, effective theory that models the behaviour...
of the system without specifying all the underlying causes that lead to system state changes; effective theories are agnostic to system mechanics (Flack, 2017). Second, mechanistic theory based on a nuanced view of the phenotype (Noble & Noble, 2023; West-Eberhard, 2003) that explicitly considers: evolutionary, ecological, developmental and physiological context; downward causation; the influence of the plant phenotype on inter-virus relations, and vice versa; the impact of timing, intensity and duration of drought interacting with viruses to modulate the plant phenotype; both the soil (moisture) and atmospheric (vapour pressure deficit) aspects of drought. Theories should scale in time, from short term to full growing season and in levels of organisation up to the relevant traits – crop yield in agriculture and fitness in nature.

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SUPPORTING INFORMATION
Additional Supporting Information may be found in the online version of this article.

Table S1. Formulas for the propagation of standard deviation (SD).

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