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4 **Effects of crop viruses on wild plants**

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20

21 **Abstract**

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23 Global land conversion and intensification of agriculture mean that remnant native plant
24 populations are increasingly exposed to crop viruses. What are the consequences for wild plants?
25 In natural unmanaged systems, the key consideration is how crop virus infection influences plant
26 fitness. Field studies of virus effects on wild plant fitness are scant. Approaches include (i)
27 observational studies, (ii) studies of experimental plants with natural infection, and (iii) studies
28 of experimental plants with experimental infection, with most studies focused on viruses in
29 the *Luteoviridae* and *Potyviridae* families. Fitness effects documented are largely neutral to
30 negative. Crop virus influence on wild plants merits attention in ecological conservation and
31 restoration.

32

33 **Introduction**

34 Humanity's appetite for food, fuel, and fiber is growing. To feed it, approximately one-half of the
35 Earth's terrestrial surface has been converted to agricultural crops and rangeland [1], and this
36 fraction will likely increase. When agriculture first developed some 10,000 years ago, cultivated
37 fields were a small fraction of the landscape, with crops surrounded by a matrix of wild
38 vegetation. Farmers, and later plant pathologists, worked hard to defend crops from what must
39 have seemed to be an overwhelmingly large natural reservoir of viruses and other pests. Now in
40 many regions the landscape balance has been reversed, with natural vegetation present in ever-
41 shrinking remnants within an expansive sea of crops and managed fields. Wildlands are regularly
42 exposed to viruliferous vectors effluxing from agroecosystems, and there is increasing evidence
43 about the potential for deleterious effects [2,3*]. Thus, a new question must be asked: What
44 influence(s) are domesticated landscapes having on plant virus ecology and evolution?
45 Specifically, what effects do crop viruses have on the fitness of wild plants? This is an urgent
46 question whose timeliness is compounded by the need to protect wild plant populations from the
47 rising threats of climate change. Unfortunately, due to a number of constraints including
48 disciplinary history [4] and biosafety logistics [3*], there is a surprising lack of empirical data. We
49 thus explore relevant published studies from the beginning of modern plant virology (1960s) to
50 the present and call for a new era of investigation.

51 **Are crop viruses pathogenic in wild plants?**

52

Most of the viruses that are central to our knowledge of plant virology are crop-associated, and were first recognized because of their presence in, and often damage to, agricultural plants[5]. We thus define “crop viruses” as those known to cause notable yield or economic loss in crops, including forage, in contrast to “wild” viruses that are associated primarily with non-crop vegetation. Many crop viruses are generalists that also can infect “wild” or unmanaged plant populations, which we define as non-cultivated plant populations in which changes in population size or genetic composition are not under direct human control [6*]. It is clear from retrospective analyses that agricultural expansion and intensification have capacity to drive changes in viral taxa, such as the proliferation of potyviruses that occurred in parallel with the first development of cropping systems [7]. Does association with crops make plant viruses more or less pathogenic? The classical hypothesis has been that agricultural association would tend to favor pathogenicity [8]. Given that crop plants represent a subset of plant life styles (mostly annual, fast growing) and environments (highly managed in modern agriculture), selective pressures might favor viruses that could propagate quickly and be readily transmitted to new hosts, characteristics associated with pathogenicity (in fact, the situation is likely more complex [8]).

Our focus here is on whether pathogenic crop viruses have consequences for wild plants. One expectation is that effects will be negative - *i.e.*, viruses pathogenic on crops will also be pathogenic on wild plants. However, plant breeding has sometimes increased crop growth rates at the expense of crop resistance[9,10], so it is possible that viruses operating in such hosts might maintain fewer defenses (*e.g.*, silencing suppressors) themselves. Thus, crop viruses might be pathogenic in crops, but have little effects on better defended wild hosts. Finally, it has also been suggested that crop viruses may provide some benefits to plants, such as tolerance to desiccation[11,12] or drought[13](but see[14]).

Quantifying virus influence on plant fitness in the field

There is much less empirical data about the effects of crop viruses on wild plants than on crop and model species. Nonetheless, there are interesting indications that virus infection in wild plants, as in crop plants, can modulate plant physiology and response to environmental resources and stresses. For example, infection increases the response of native grasses to resource release with simulated grazing [15]. Evaluation of the molecular and physiology mechanisms of wild plant – crop virus interactions is thus valuable. Here we wish to highlight the critical need for investigation of virus effects at the whole plant level, namely effects on wild plant fitness in the field. “Fitness” is an evolutionary biology term that describes how successfully an individual propagates its genes [16]. We thus use the term “fitness” to mean the contribution of an individual, in terms of offspring, to the next generation [17]. Fitness is a cumulative term that integrates plant survival and reproduction at different life stages (Fig. 1A). It is distinct from “yield,” which quantifies commodity production by a population of plants in a given area (*e.g.*, bushels of maize harvested per acre). Fitness is of central importance in plant ecology because it is a key determinant of plant population and community dynamics, as well as plant evolution (Fig. 1B).

To evaluate virus effects, attention is often paid to symptoms[8], such as distinctive discoloration or abnormal morphology, and to growth measurements, such as plant height or biomass production. All of these metrics have value. To quantify virus effects on fitness, however, what is most needed are measures of individual fitness components across the plant life cycle including survivorship to specific life stages, and metrics of reproduction such as probability of flowering and quantity of seed produced (Fig 1A). These metrics are useful on their own and gain even more power when integrated by models to estimate overall fitness effects,

using approaches such as matrix modelling [18*,19*] and aster models [17]. For wild plants, measures of fitness components are best obtained in outdoor field settings where individuals experience fluctuations in weather and resource availability, natural disturbances, and true biologically active soils with sufficient rooting depth. In plant science, it is increasingly recognized that pots can cause growth artefacts [20] and that plants grown in static, controlled situations may not manifest the full range of phenotypes evident in dynamic environments[21]typical of natural settings. Thus, the most convincing documentation of crop virus effects on wild plants is likely to come from studies of fitness effects in the field. Such studies typically employ one of three different approaches and require consideration of multiple issues, including biosafety requirements and interpretation of causality (Box 1 and graphical abstract).

Crop virus influence on wild plant fitness components is largely negative to neutral

To summarize current understanding, we surveyed the ecological and virological literature. Because field studies of virus effects on wild plant fitness are scant, we includednaturalized pasture species in our definition of “wild” plants and searched as far back as the 1960’s when plant virus detection methods first began to modernize. In17publications, we found reports of 20 studies that examined the effect of crop viruses of any type on fitness or fitness components of wild plants in the field. Some individual publications described several studies each [18,22,23*]; in another case, we counted two separate publications as different time points of a single longer-term study[24,25]. Most of the studies examinedeither *Barley and Cereal yellow dwarf viruses* (genera*Luteovirus*and *Polerovirus*, family *Luteoviridae*: B/CYDVs) in grasses, which have become a model system in plant virus ecology, or potyvirus(es)(family

Potyviridae); a few crop viruses in the *Bromoviridae*, *Geminiviridae*, *Secoviridae*, and *Tymoviridae* families were also considered (Table 1).

The field studies were conducted in temperate or Mediterranean climate systems in Australia, Japan, the UK, and the USA (Table 1). Most studies were just 1-2 yrs in length with two longer studies [23-25]. Each study quantified at least one fitness component, sometimes several, and two presented integrated measures of fitness [18,19]. Many studies also quantified growth metrics. About 1/3 of the studies examined annual plants, the rest perennials (including biennials). Even among this set of “wild” plants, however, all of the subjects were herbaceous species from families well-represented in agriculture, and 40% of the subject groups represented species exotic to the study region.

Across all study types, the documented effects of crop viruses on fitness components of wild plants were largely negative to neutral (Fig. 2). There was no qualitative difference in this tendency across the six crop virus families represented. About one-half of the studies (9/20) measured crop virus effects on host survivorship with 89% (8/9) reporting negative effects on some or all host genotypes. Of the 19 studies that reported measures of reproductive success, 84% (16/19) observed only negative to neutral effects, with nearly one-half (9/19) reporting solely negative effects. Negative reproductive effects associated with infection included reduction in probability of flowering [22], inflorescence number [23*,26,27], reproductive biomass [28*], numbers of fruits and of male flowers [29], and overall seed production or fecundity [15,19*,22,23*,25]. Growth metrics, while not direct measures of fitness, also were negatively associated with infection in 88% (13/15) of the studies reporting them. Negative growth effects included reductions in leaf number [22,30]; height [15,22,31-33*]; tiller number [15,27], and growth rate, vigor, or overall biomass production [15,19*,23*,25,28*,33*-35].

Only a few studies found positive effects of crop virus infection on wild plant fitness in the field. Prendeville et al. [18*] reported that in fitness models inoculation with *Zucchini yellow mosaic virus* (genus *Potyvirus*, family *Potyviridae*) increased population growth rate in one of three populations of an annual gourd, although significant differences were not evident in individual fitness components. In other cases, infection had positive effects on plant reproduction or phenology but not survivorship. In a survey of annual weedy grasses (Method 1), for example, the panicle length of *Setaria pumilla* ssp. *pumilla* was positively associated with luteovirus infection in one of two years [33*]. In our review, observational studies (Method 1, Box 1) had a somewhat greater proportion of neutral to positive results, perhaps because of non-random processes in infection acquisition, or because the logistics of more controlled experiments (Method 3) favor inoculation of young plants that in turn may show greater responses to inoculation than mature individuals. In three other studies, virus infection altered plants traits in ways that might increase fitness. However, given the overall results of each study, evidence of increased fitness was not readily apparent. In the first example, *Turnip mosaic virus* (genus *Potyvirus*, family *Potyviridae*) infection reduced flea beetle damage in wild *Brassica oleracea* [27], but fungal damage rose, and seed production and survivorship were reduced. In the second example, Catherall [20] found that *Barley yellow dwarf virus* infection stimulated early growth, tillering, and spring biomass production in mowed *Lolium perenne*. However, this virus-induced shift in phenology likely did not increase host fitness because infected plants produced less total herbage and the ratio of fertile to vegetative tillers was reduced. Moreover, in communities with clover, virus infection in *Lolium* favored clover production. In the third example, Mackenzie reported that infected *Primula* tended to bloom earlier [30]; we counted this as a positive fitness effect, but it might not be if, for example, this timing is not optimal for

pollinators. Our findings of limited positive effects of crop virus infection contrast with notable positive effects on host survivorship under herbivory in a rare study of wild plants with wild virus infection[36]. Overall, our summary supports concerns raised by recent greenhouse inoculation trials that crop viruses have substantial potential to harm natural plant populations [3*].

Conclusions

Increased understanding of the effects of crop viruses on wild vegetation is important for two reasons. First, wild plants are tremendously diverse in terms of morphology, physiology, and life cycle. By understanding how viruses interact with these species, we will gain a broader understanding of plant-virus interactions in general. Second, wild plants are increasingly limited to habitat patches that are surrounded by an agricultural matrix, and thus have increased exposure to crop viruses. Such viruses thus deserve attention from a conservation perspective.

Our focus was to explore the effects of crop viruses on plant fitness, given that fitness measurements are central for ecological and evolutionary investigations. We found that most reported effects of crop viruses on wild plants are neutral or negative. The number of available studies is, however, limited. To overcome past biases, new studies need to be done with (1) a wide diversity of types of viruses, (2) a wide diversity of types of plants, and (3) in the context of diverse environments. The latter is particularly important since there is clearly not a single effect of viruses on plant fitness, but likely a range of effects depending on environmental context.

We thus advocate for a new generation of field studies of virus effects on plant fitness. For those viruses for which field experiments are feasible, we suggest field factorial experiments where virus-inoculated and mock-inoculated plants are grown, for example, with reduced vs.

enhanced water treatments or with exposure to herbivores vs. protection from herbivores (e.g., via cages, insecticides). Such environmental manipulations are commonly done by plant ecologists. It is now time to integrate such ecological approaches with questions in virology.

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CAPTIONS

Table 1. Field studies of effects of crop viruses on wild plant species. For Study Methods, see Box 1. Host types: EA – exotic annual; EP – exotic perennial; NA – native annual; NP – native perennial. *Biennial.

Box 1. Methodological approaches for studying virus effects on wild plant fitness in the field.

Figure 1.(A) Fitness components. The lifetime fitness of an individual plant depends on fitness components including its probabilities of survival to different life stages (s_0 , s_1 , s_2), and, if it survives, on the probability that it flowers (fp), the number of flowers it produces (nf), and the number of seeds it produces per flower (fs) at each age (two-year life cycle shown). Virus infection, whether naturally occurring or experimentally induced, can occur at different points in the life cycle. For example, experimental inoculations can be performed on seedlings [23] or on mature plants [18]. The effect of the virus on plant fitness may depend on the timing of infection relative to plant life history events. (Note: We used a perennial sunflower as the focus of this figure; for this species, each inflorescence (“head”) consists of many flowers). **(B) Fitness as a central mediator of effects in plant ecology and evolution.** Virus infection can affect plant fitness (either negatively or positively), which in turn can alter plant population dynamics (changes in plant numbers over time), plant evolution, or plant community dynamics (changes in plant species composition over time). These three processes can in turn alter patterns of virus

infection. For example, virus infection could decrease plant fitness, and if there is genetic variation in susceptibility, plants may evolve to become more resistant. Greater resistance in the plant population may reduce rates of virus infection (and possibly lead to evolutionary changes in the virus). Note that to keep the figure simple, we have not included arrows between the ovals for plant population dynamics, evolution, and community dynamics, even though these processes certainly interact.

Figure 2. Observed effects of crop virus infection on wild plant species. For Study Methods, see Box 1. Host types: EA – exotic annual; EP – exotic perennial; NA – native annual; NP – native perennial. *Biennial. Effect codes: –, negative; 0, neutral; +, positive. For some measures, more than one code is given, reflecting multiple treatments, plant taxa, viruses, or measurement time points. Virus species studied include CMV (*Cucumber mosaic virus*, genus *Cucumovirus*, family *Bromoviridae*), TLCV (*Tomato leafcurl virus*, genus *Begomovirus*, family *Geminiviridae*), B/CYDV (*Barley and Cereal yellow dwarf viruses*, genera *Luteovirus* and *Polerovirus*, family *Luteoviridae*), BYMV (*Bean yellow mosaic virus*, genus *Potyvirus*, family *Potyviridae*), CIYVV (*Clover yellow vein virus*, genus *Potyvirus*, family *Potyviridae*), CSV (*Cocksfoot streak virus*, genus *Potyvirus*, family *Potyviridae*), TuMV (*Turnip mosaic virus*, genus *Potyvirus*, family *Potyviridae*), ZYMV (*Zucchini yellow mosaic virus*, genus *Potyvirus*, family *Potyviridae*), ArMV (*Arabidopsis mosaic virus*, genus *Nepovirus*, family *Secoviridae*), TRSV (*Tobacco ringspot virus*, genus *Nepovirus*, family *Secoviridae*), and TYMV (*Turnip yellow mosaic virus*, genus *Tymovirus*, family *Tymoviridae*).

Box 1. Methodological approaches for studying virus effects on wild plant fitness in the field.

Method 1. Natural plant populations with naturally acquired infection

Fitness components of naturally established plants exposed to natural infection are measured and related to their infection status [33]

Advantages

- Describes ecologically relevant virus interactions in natural vegetation
- No need to directly manipulate viruses; few permitting requirements.
- Avoids cost of managing experimental plantings; feasible to survey large area.

Disadvantages

- Difficult to interpret causality (*e.g.*, EW Seabloom, et al. [37]). Non-random virus acquisition is a particular challenge. For example, if vectors prefer larger [or smaller] plants, infection status may be associated with size because of transmission dynamics and not necessarily because infection alters plant growth.
- Confounding variability may be introduced if plants gain infection at different life stages or become co-infected with multiple viruses.
- Mortality of young plants may be hard to detect.

Method 2. Experimental plant populations with naturally acquired infection

Experimental plant populations with known properties are established in the field and allowed to acquire natural infection from the environment. Plants might be clones (*e.g.*, modifying the method of S Mackenzie [30]) or virus-susceptible and virus-resistant lines that are otherwise similar, such as in transgenic populations [29].

Advantages

- Greater control of plant material facilitates interpretation of results
- Preserves natural infection process; usually permits not required unless GMO.
- Effective for testing questions about factors controlling infection rates and patterns of virus accumulation over time

Disadvantages

- Natural infection rates may be too low or too high to achieve experimental goals,
- Confounding variability may be introduced through process of natural inoculation.

Method 3. Experimental plant populations with experimental infection

Individual experimental plants or plant communities are directly inoculated or mock-inoculated with a known virus. For vector-borne viruses, individual plants are typically infected indoors to contain vector movement, and then are treated with pesticide and transplanted outdoors. For mechanically transmitted viruses, field inoculation may be more feasible [18]. Depending on biosafety requirements, efforts may [23] or may not [22] be required to limit subsequent vector contact with plants. Alternatively, for community-level experiments, viruliferous and non-

viruliferous vectors may be released within caged field plots for a determined time period and then killed [28].

Advantages

- Strongest means of quantifying direct, causal relationships between virus infection and host plant fitness.

Disadvantages

- Biosafety concerns limit studies to specific low-threat viruses or environments.
- Logistically demanding; often favor experimental designs in which plants are inoculated when young and most easily handled.

Method 4. Natural plant populations with experimental infection.

This fourth method has not been used, to our knowledge. Given that it would address causal relationships between infection and plant fitness in the context of naturally growing vegetation, this approach would most directly explore whether infection alters plant fitness field populations, Variability within natural populations may, however, make results difficult to interpret.

Table 1

Table 1. Field studies of effects of crop viruses on fitness or fitness components of wild plant species. For Study Method, see Box 1. Host types: EA – exotic annual; EP – exotic perennial; NA – native annual; NP – native perennial. *Biennial.

Virus Family & Species	Host Type	Host Genus or Genera and Study Conditions & reference	Study Method	Ref.
Bromoviridae CMV ¹	NA	<i>Cucurbita</i> ; 1-yr study in Mississippi (USA) field	3	[16]
Geminiviridae TLCV ²	NP	<i>Eupatorium</i> ; shaded and gap sites in Japanese forest.	1	[21, 22]
Luteoviridae B/CYDV ³	EA	<i>Bromus</i> , <i>Setaria</i> ; survey of experimental oat plot, New York (USA)	1	[31]
	EA	<i>Avena</i> , <i>Bromus</i> ; survey of 18 grasslands along North American west coast	1	[35]
	NP	<i>Festuca</i> ; 1-yr study of restoration planting in California (USA) field	2	[20]
	NP	<i>Lolium</i> ; 2-yr field study of individuals and simulated swards with clover and clipping, Wales (UK)	3	[29]
	EP	<i>Anthoxanthum</i> ; 1-yr study in mown field, North Carolina (USA)	3	[25]
	EA	<i>Avena</i> , <i>Digitaria</i> , <i>Lolium</i> , <i>Setaria</i> ; 1-yr study in New York (USA) of caged experimental communities	3	[26]
	NP	<i>Elymus</i> , <i>Festuca</i> , <i>Hordeum</i> , <i>Koeleria</i> , <i>Nassella (Stipa)</i> ; 3-yr study in California (USA) field of individuals from multiple populations.	3	[20]
	EA	<i>Bromus</i> ; 1-yr study California (USA)	3	[20]
	NP	<i>Elymus</i> , <i>Nassella (Stipa)</i> ; 1-yr study in California (USA) ag. field, with competition & clipping	3	[13]
Potyviridae BYMV ⁴	EP	<i>Trifolium</i> ; planting in Minnesota (USA) pasture	2	[24]
CIYVV ⁵	EP	<i>Trifolium</i> ; 18-month study at grassland & woodland sites in south-east Australia, with competition in grassland site	3	[17]
CSV ⁶	NP	<i>Dactylis</i> ; ca. 18-month study of artificial swards, with clipping, Wales (UK)	3	[32]
TuMV ⁷	NP*	<i>Brassica</i> ; 2-yr-study in Dorset (UK) fallow field	3	[23]
ZYMV ⁸	NA	<i>Cucurbita</i> ; Pennsylvania (USA) ag. field, with cucumber beetles & bacterial infection	2	[27]
	NA	<i>Cucurbita</i> ; 1-yr study in Mississippi (USA) field	3	[16]
Secoviridae ArMV ⁹	NP	<i>Primula</i> ; five sites with natural populations in Wales (UK)	2	[28]
TRSV ¹⁰	EP	<i>Lotus</i> ; 1- to 2-yr field study in Maryland (USA)	3	[33]
Tymoviridae TYMV ¹¹	NP*	<i>Brassica</i> ; 2-yr-study in Dorset (UK) fallow field	3	[23]

¹Cucumber mosaic virus, ²Tomato leaf curl virus, ³Barley and Cereal yellow dwarf viruses, ⁴Bean yellow mosaic virus, ⁵Clover yellow vein virus, ⁶Cocksfoot streak virus, ⁷Turnip mosaic virus, ⁸Zucchini yellow mosaic virus, ⁹Arabis mosaic virus, ¹⁰Tobacco ringspot virus, and ¹¹Turnip yellow mosaic virus

Figure 1

Figure 2

Virus study						Fitness components												Growth metrics						
Virus family	Virus	Method	Host Type	Host Genus	Ref #	Survivorship	Early bloom	Prob. flowering	No. flowers	No. flower heads	No. flowers per head	No. reprod. tillers	Panicle length	No. fruit or pods	Reproductive biomass	Seeds per fruit	Fecundity, seed prod. or mass	Basal area	Height	Leaf area	No. leaves	Tiller no.	Growth rate, vigor	Biomass
Bromoviridae	CMV	3	NA	Cucurbita	18									0		0								
Geminiviridae	TLCV	1	NP	Eupatorium	24, 25	−		0									−		−				−	
Luteoviridae	B/CYDV	1	EA	Poaceae spp.	33							− +	−0+						0			+		−0
	B/CYDV	1	EA	Poaceae spp.	37											0								
	B/CYDV	2	NP	Festuca	23				−								−	0						0
	B/CYDV	3	NP	Lolium	31	0	+												−			0+		− +
	B/CYDV	3	EP	Anthoxanthum	27				−													−		
	B/CYDV	3	EA	Poaceae spp.	28										−0									−0
	B/CYDV	3	NP	Poaceae spp.	23	−0						−0					−0	−0						−0
	B/CYDV	3	EA	Bromus	23												−							−
	B/CYDV	3	NP	Poaceae spp.	15	−											−	−	−			−		−
Potyviridae	BYMV	2	EP	Trifolium	26				−	−														
	CIYVV	3	EP	Trifolium	19	−											−						−	
	CSV	3	NP	Dactylis	34	−																		−
	TuMV	3	NP*	Brassica	22	−		0						−			−		0	0	0			
	ZYMV	2	NA	Cucurbita	29				−					−										
	ZYMV	3	NA	Cucurbita	18									0		0								
Secoviridae	ArMV	2	NP	Primula	30		+		0												−			
	TRSV	3	EP	Lotus	35	−0			−	−0						−	−						−	
Tymoviridae	TYMV	3	NP*	Brassica	22	−		−						−			−		−	−	−			

Field methods of studying virus effects on plant fitness