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Current Opinion in Virology

Viruses in the phytobiome

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The phytobiome, defined as plants and all the entities that interact with them, is rich in viruses, but with the exception of plant viruses of crop plants, most of the phytobiome viruses remain very understudied. This review focuses on the neglected portions of the phytobiome, including viruses of other microbes interacting with plants, viruses in the soil, viruses of wild plants, and relationships between viruses and the vectors of plant viruses.

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Current Opinion in Virology 2019, **37**:72–76

This review comes from a themed issue on **Viruses and the microbiome**

Edited by **Stephanie M Karst** and **Christiane E Wobus**

For a complete overview see the Issue and the Editorial

Available online 13th July 2019

https://doi.org/10.1016/j.coviro.2019.06.008

Introduction

The phytobiome is defined as 'plants, their environment, and their associated communities of organisms' [1]. This includes weather forces, soil and communities living in the soil, insects and grazing animals and communities living in these animals, and microbes colonizing plants directly (Figure 1). Examining viruses in this rather broad reach of organisms could potentially include viromes of all terrestrial life, and perhaps marine life too if we include marine plants. However, this short review will limit the phytobiome to focus on the viruses of terrestrial plants and the microbes that directly interact with plants in either the above ground or below ground tissues, with a brief discussion on the three-way interactions among viruses and the insects that feed on plants. Recent reviews have covered some aspects of viruses in the phytobiome, so the focus of this paper is on the less studied portions of the phytobiome viruses.

Viruses are probably the most neglected microbes of the phytobiome. There are a number of reasons for this: it is more difficult to screen for viruses because they lack any signature genes with enough sequence similarity to be detected by PCR or RT-PCR; traditional virology has focused on pathogenic viruses; and very little work has been done outside of crop plants. In the past decade a number of deep sequencing studies have begun to address the incidence and diversity in viruses of plants [2•], but there is still limited information on viruses of wild plants, and even less about viruses in soil communities or viruses of bacteria and fungi within plants.

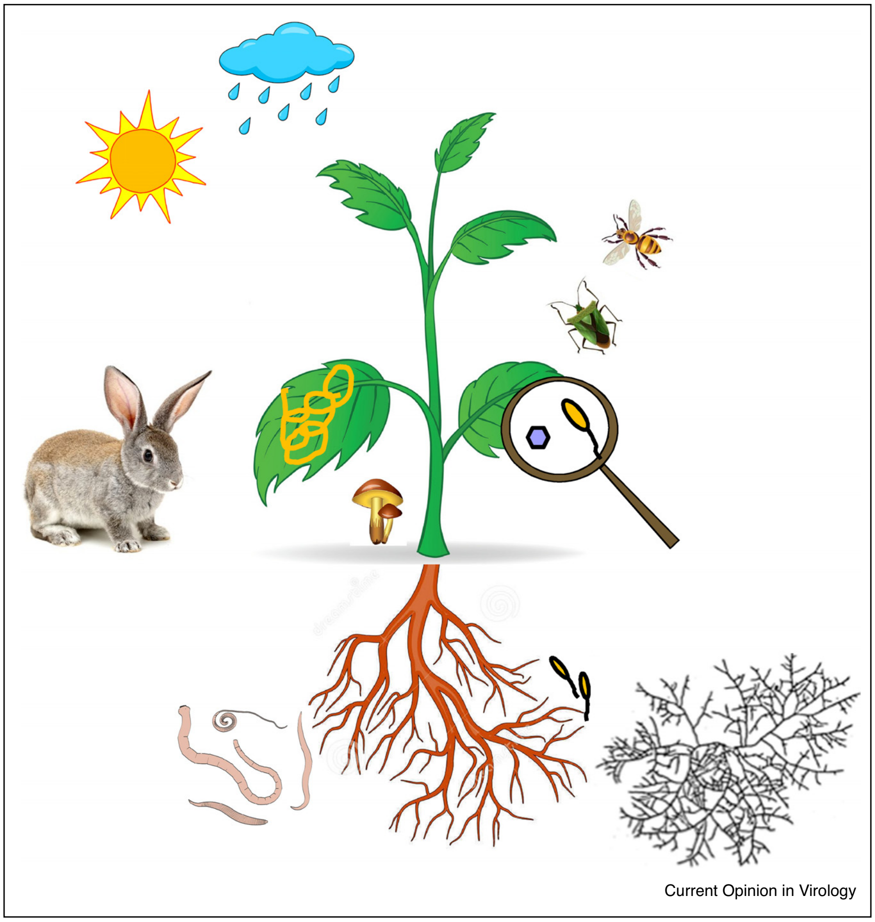
A recent review of the role of viruses in phytobiomes covers many aspects of these interactions in depth [3••]. Here I will provide an overview of what is known, and discuss some aspects that are less well covered in this recent review.

Viruses that are beneficial to plants

Knowledge about viruses in the phytobiome is restricted by several biases: for more than a century most studies of viruses have focused on pathogens, even though beneficial viruses are found in all environments [4,5]; most studies have focused on experimental systems that are easy to manipulate but probably don't reflect the scope of viruses in nature; and, virus research has been very human-centric, so for plants studies have focused almost exclusively on crop plants.

One of the first studies of a beneficial phytobiome virus was the virus-infected fungus that confers thermal tolerance in geothermic soils in Yellowstone National Park [6•]. In this system plants growing in soil temperatures above 50°C are colonized by a fungus, *Curvularia protuberata*, that is in turn infected with Curvularia thermal tolerance virus. All three members of this holobiont are required for success in this hostile environment [6•]. Understanding the molecular details of this interaction has been hampered by the difficulties in studying multitrophic interactions, but studies of the virus–fungus interactions in culture have implied that the pigment melanin and the sugar trehalose are involved [7]. Other beneficial viruses have been found in plants, including several acute plant viruses that confer drought tolerance or cold tolerance [8], and in several cases virus infection of plants in field studies has shown an advantage under drought conditions [9,10]. Many plant viruses are involved in interactions between insects and plants (see below), in most cases benefitting the virus by enhancing transmission, but some viruses deter insects that carry other diseases [11] and in a recent study a persistent plant virus in Jalapeña peppers deterred aphids by volatile emissions, and reduced the quality of the plants as a host for aphids [12•]. In legumes the coat protein gene of another persistent virus regulates nodulation by suppressing the

Figure 1



The phytobiome.
Microbes of the phytobiome include not only plant viruses, but the viruses of everything that interacts with plants, including other microbes, insects and other invertebrates, other plants including parasitic plants such as dodder, and herbivorous animals.

formation of nodules when adequate nitrogen is available from the soil [13].

Viruses of plant pathogenic fungi

Viruses of microbes that interact with plants are poorly studied, although fungal viruses have received more attention than other microbe viruses [14]. Fungi have varied interactions with plants, a majority of which are positive (known as endophytes). Many endophytes have viruses, but, with a few exceptions, the roles they play in plant–fungal interactions are largely unknown [15]. Plant pathogenic fungi are more thoroughly studied than beneficial endophytes, even though they are less common, and the roles of viruses in reducing the virulence of plant pathogenic fungi, a phenomenon known as hypovirulence, has been most thoroughly studied in *Cryphonectria parasitica*, the causal agent of chestnut blight. Chestnut blight devastated the vast chestnut forest of the northeastern United States in the early 20th century, and the stately tress have all but disappeared. Cryphonectria hypovirus 1 (CHV1) reduces the disease and allows trees to survive [16]. Fungal viruses are not readily transmitted among fungi, and in general transmission only occurs between closely related fungal strains that share vegetative incompatibility (*vic*) genes, through anastomosis [17]. However, transmission of fungal viruses is a poorly studied area, and recent studies have suggested other means of horizontal transmission in addition to anastomosis [18,19].

Using CHV1 to control chestnut blight has not been successful in North America due to the diversity of strains, but has been successful in Europe where there are fewer *vic* groups [20]. A recent strategy to modify *C. parasitica* to allow broader transmission of CHV1 holds some promise of restoring chestnut forests in North America [21••]. *C. parasitica* hosts a number of additional diverse viruses as well as CHV1, and a number of other fungal hypoviruses have been identified in a broad range of plant pathogenic fungi [14]. These viruses could be considered beneficial to plants, but they are not usually beneficial to their fungal hosts.

A direct role of viruses in plant fungal pathology has not been explored to any extent, but in one example a fungal pathogen of pear showed reduced growth in culture when infected with a virus [22], but enhanced pathology due to the production of a toxin [23•]. It seems likely that similar scenarios exist with other fungal pathogens.

Viruses of plant-associated bacteria

Bacteriophages can be lytic, meaning they are transmitted horizontally and usually kill their hosts, or lysogenic, meaning they exist as prophage within the host genome. Phages occasionally switch between these lifestyles depending conditions. In human pathogenic bacteria a majority of the pathogenicity factors are encoded by lysogenic bacteriophages [24].

Bacteriophages in the plant can be divided into phyllosphere viruses and rhizosphere viruses. In plant pathogenic bacteria the role of phages in plant disease has been less studied [25], but searching the genomes of plant-associated bacteria demonstrated that a large number of phage-derived genes are present in these bacteria [26]. In a few cases virulence factors have been linked to prophage sequences, including in *Psuedomonas*, *Xanthamonas* and *Xylella* [26], bacterial agents of a variety of plant diseases. Phages are also responsible for horizontal gene transfer that could potentially convert non-pathogenic bacteria into a pathogen through acquisition of virulence factors.

The discovery of lytic phage that are lethal to plant pathogenic bacteria dates to the early days of phage research [27•]. A number of studies have looked at the possibility that phages could be used to control plant diseases (Table 1) but in most cases these have not held up well in field experiments [28]. There is intensified research in this area due to the increasing impact of antibiotic resistance in animal and human health, and there have been recent attempts to engineer viruses to be more effective biocontrol agents for fireblight [29], even though there is no evidence that antibiotic use in crops, primarily in treatment of fireblight, has any negative impacts [30].

The role of phages in the phyllosphere bacterial abundance and diversity in plants has been assessed in an experimental study where microbial populations from tomato were depleted of viruses and then inoculated onto young tomato seedlings. When the viruses were added back the abundance and diversity of bacteria was altered, indicating that phages play a role in regulating the bacterial populations in plants [31••].

Table 1

Experimental plant bacterial disease control with bacteriophage[a]

Disease	Causative bacterial agent
Cabbage rot	*Xanthomonas campestris* pv. *campestris*
Soft rot of potato	*Pectobacterium atrosepticum*
Stewart's wilt of corn	*Pantoea stewartii*
Soft rot/blackleg of potato	*Dickeya solani*, *Pectobacterium spp.*
Bacterial wilt of tomato	*Ralstonia solanacearum*
Kiwifruit canker	*Pseudomonas syringae* pv. *actinidae*
Bacterial blight of leak	*Pseudomonas syringae* pv. *porri*
Citrus bacterial spot	*Xanthomonas axonopodis* pv. *citrumelo*
Citrus canker	*Xanthomonas axonopodis* pv. *citri*
Pierce's disease of grape	*Xylella fastidiosa*
Apple/pear fire blight	*Erwinia amylovora*

[a] Taken from Ref. [28].

As with fungi, many phyllosphere bacteria are beneficial. The most well studied are the growth-promoting bacteria [32]. However, viruses of these bacteria have not been studied in any depth.

In the rhizosphere metagenomic studies indicate that viruses are abundant, but again this is an understudied area of research, and soil viruses are very poorly studied. Most are believed to be bacteriophages, although there are likely viruses from other sources as well, including archeal viruses, fungal and nematode viruses from plant-interacting eukaryotes, and plant roots also may release viruses into the soil, but studies to date have only investigated phages [33•].

Early studies on soil virus abundance used plaque assays, but this is a very limited assessment, because it will only detect viruses that infect the specific host used in the assay. Direct counts of viruses by epifluorescence microscopy or transmission electron microscopy have revealed estimates of gross counts of virus particles in soil samples. A majority of soil virus abundance studies have not looked at agricultural soils [33•]. A common measure of the role of viruses in the ecology of bacterial communities is the virus:prokaryote ratio, or VPR. A high VPR is taken to mean that most viruses in the sample are lytic rather than lysogenic. In an analysis of published studies on phage abundance, the VPR in soils is extremely high compared to other environments; however, this varies widely and includes very few studies [34]. Analyzing 5 TB of metagenomic data from numerous studies, 125 000 partial virus sequences were identified, and CRISPR sequences were used to link some of these to hosts. The soil terrestrial viruses were one of the smallest pieces of the pie [34]. Although some metagenomic studies have suggested that there are also single-stranded filamentous phages in soil environments, these have been largely neglected in soil studies [35].

The most well-studied rhizosphere bacteria are the symbiotic rhizobia that colonize the roots of legumes and are involved in nitrogen fixation. Hundreds of phages have been identified in rhizobia, and there is a wide variation in the susceptibility of rhizobia to lytic phages. Jaiswal and Dakora proposed using susceptibility to rhizobiaphage as a tool to select appropriate bacteria for symbiotic interactions in agricultural legumes [36].

Plant viruses

Tobacco mosaic virus was the first viruses described in the late 19th century [37•]. Since then thousands of plant viruses have been identified and characterized [38], but the vast majority have been viruses of crops. In recent years a few studies have begun to characterize viruses of wild plants using metagenomic studies [2•,39], and there have been some important studies on how viruses move across the agro-wildland border [40,41••]. The acute viruses are generally asymptomatic in the wild plants, but can induce symptoms in experimental studies in greenhouse plants [42]. An interesting aspect of studies in wild plants is that a majority of viruses are from the persistent virus families that are generally asymptomatic [43]. These virus families are also common in crop plants, but are very understudied, probably due to the lack of any evidence of pathogenicity, and the difficulty of studying them due to very low titers [44]. Many of the persistent plant viruses have relatives that infect fungi, and recently some well-known acute plant viruses have been described infecting fungi [45].

Plants, viruses, and vectors

A majority of acute plant viruses are transmitted by insects. Some of these viruses also infect insects, but the majority do not. In recent years a number of studies have looked at the impacts that plant viruses have on their insect vectors, including manipulating plant volatiles to attract vectors, and manipulating anti-feeding compounds to encourage vectors to move to new plants once the virus has been acquired [46].

Nematodes are known to vector a number of plant viruses, but nematode viruses had not been studied until recently with the discovery of a virus in a wild isolate of *Caenorhabditis elegans* [47]. This led the way to further studies, and a number of viruses have been described in plant-pathogenic nematodes [48]. The impact of nematode viruses on the nematodes or the plants they interact with is as yet unknown.

Another common vector of plant viruses are the parasitic plants known as dodder [49]. These plants can be infected with the viruses they transmit, but there has been very little work done in this system in recent years.

Conclusions

Viruses in the phytobiome offer a rich area for basic research in ecology, as well as the discovery of many relationships that may be utilized in beneficial ways for agriculture or in bioremediation. For the most part the viruses in the phytobiome are very understudied, with some areas such as the soil virome and beneficial viruses especially neglected.

Conflict of interest statement

Nothing declared.

Acknowledgements

This work was supported in part by the USDA National Institute of Food and Federal Appropriations under Project PEN04652 and Accession number 1016233.

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- •• of outstanding interest

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