Spring 2018

The under-appreciated roles of viruses in agroecosystem management

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The under-appreciated roles of viruses in agroecosystem management

by

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A creative component submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

Major: Agronomy

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Iowa State University
Ames, Iowa
2018

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Abstract

Increasing food demand and diminishing resources require food production practices to become more productive with fewer external inputs. Agroecology uses ecological principles to inform agroecosystem management that results in ecosystem services replacing external inputs to the system and an economically and environmentally sustainable system. Viruses have historically been ignored in ecological studies. Understanding the ecological role of viruses in agroecosystems is key to managing crop production systems that utilize ecosystem services rather than rely on external inputs. The role of viruses in microorganism communities, where most research has been conducted, provides insight into the ecological role of viruses in other systems. Research in plant communities indicates viruses also have a substantial role. While it is becoming clear that most viruses are asymptomatic, some have been identified that have beneficial effects on their plant hosts. Recent advances in virus detection are rapidly expanding the field of virus ecology, with implications for agroecology as the practice develops.
I. Introduction

In a recent report, the United Nations (UN) estimates that the current world population of 7.3 billion will increase to 9.8 billion by 2050 (United Nations, 2017). Agriculture production systems are faced with the challenge of increasing food production to meet the growing need while conserving limited resources. Resources required for crop production are not only finite, but conventional agriculture practices galvanized by the green revolution have serious consequences for the environment. Environmental degradation and pollution mean that the food production gains made since the green revolution cannot be sustained using the same approach, i.e., maximizing short-term yields and profits through chemical intensification and system simplification (Weiner, 2003; Tilman et al., 2011; Malézieux, 2012a; Pimentel and Burgess, 2014). A new approach based on knowledge of ecological processes and provision of ecosystem services is necessary to create economically and environmentally sustainable crop production systems capable of meeting food demand. Research is proving that the same or higher yields can be achieved through ecologically based management that requires fewer inputs which diminish our resource base (Davis et al., 2012).

A substantial proportion of the world’s population remains malnourished or undernourished, though the proportion has dropped in recent decades (FAO et al., 2015). Reasons for pervasive food shortage go beyond lack of abundance and include poverty, harmful economic systems, conflict, food and agriculture policies, and climate change (World Hunger Education Service, 2016). Agriculture production has risen substantially in the last fifty to sixty years, primarily due to advancements during the green revolution, though incremental gains are diminishing (Pimental and Wilson, 2004). Fundamental
scientific advancements during the green revolution were genetic improvement of crops, especially high-yielding varieties and faster maturity (Pingali, 2012), continued development of farm machinery and related technology, and widespread development and distribution of synthetic fertilizers and pesticides. As a result, while world population doubled over the last 50 years, cereal crop production tripled while land under cultivation increased by only 30% (Wik et al., 2008). Yields in developing countries have increased substantially. For example, the yield per hectare of wheat increased 208%, rice production increased 109%, maize saw an increase of 157%, and potato and cassava production rose 78% and 37%, respectively (FAO 2004, Pingali 2012). Though these gains were not distributed equally across countries and crops (United Nations, 2017), they reflect the contributions of agriculture producers and the related scientific community to address global needs for increased food production.

These gains have not come without substantial costs. The amount of grain produced per capita has continually decreased since 1984, which is linked to a decline in per capita cropland largely as a result of soil degradation and aquifer depletion (Pimental and Wilson, 2004). Sediment from tillage and erosion is the number one pollutant of surface waters (Jang et al., 2013), and loss of wildlife habitat and biological diversity as a result of agriculture expansion and intensification (Feather et al., 1999) is a serious concern for maintaining crop productivity over the long term. Consequences of agriculture production on global systems include marine eutrophication, desertification, species and habitat loss, air pollution, and contributions to the acceleration of global warming (Duru et al., 2015). Regional effects of conventional management practices on the environment include local water pollution, development of genetic or behavioral changes among target
species populations (Thrall et al., 2011), soil degradation, and soil erosion. While the impressive gains as a result of the green revolution have generally resulted in increased global food security, it is becoming clear that productivity levels cannot be sustained by using the tools provided by the green revolution alone. As the returns from advancements during the green revolution are diminishing and deleterious effects are becoming clear, it is time to incorporate ecological principles into management of crop production systems in order to increase productivity while conserving to the fullest extent our resource base.

Agroecology bases the design and management of agroecosystems on ecological principles, which then enables external inputs to be replaced by provision of ecosystem services as a result of ecological processes (Nicholls et al., 2016). Viruses have long been recognized as potential threats to realizing yield potential, but their ecological role has typically been ignored. As scientific advancements in virus detection improve, especially next-generation sequencing, we are learning that viruses have more to contribute to ecosystems than disease. Numerous viruses have been identified in only the past few years that have beneficial effects on their plant hosts. A synthesis of the current literature available on known, beneficial effects of some plant viruses on their hosts is important to more fully understand and evaluate the potential role of viruses in crop production, especially as it pertains to maintaining long-term productivity of agroecosystems.
II. Agroecology

In 1939, Herbert Hanson, sitting president of the Ecological Society of America, implored fellow ecologists at the society’s annual meeting to conduct research in cropping systems in order to, “ferret out relationships with the environment so that man, using this knowledge in conjunction with that obtained from other fields, can strive intelligently to secure balance and stabilization” (Hanson, 1939). The field of agroecology has developed with this aim, invigorated by the environmental and societal consequences of crop production systems developed since the green revolution (Wezel et al., 2009). Wezel et al (2009) point out that the term ‘agroecology’ is used with three distinct references: agroecology as a science, a political or societal movement, and as an agricultural practice. Scales of study range from the plot and field level to the agroecosystem and farm level to the level of global food production systems.

This summary focuses on agroecology as an agricultural practice at the field and agroecosystem levels. Decisions made at the farm level contribute to global food production systems, but ‘agroecology’ defined as the “ecology of food systems” (Francis et al., 2003) includes global societal and economic aspects that are not considered here. The working definition for this paper is based on earlier, but still widely used, descriptions of ‘agroecology’ as an “an applied ecology to plant production and agricultural land management” (Hénin, 1967; Wezel et al., 2009) with a focus on how ecological principles function within crop production systems and how they can be applied to solve problems therein (Jackson, 1997). Cropping practices based on ecological processes and provision of ecosystem services are essential to meet increasing
food demand sustainably with respect to the environment and the economic security of farmers (Wezel et al., 2014).

A. Agroecosystems

An ecosystem is a “dynamic complex of plant, animal, and microorganism communities and the nonliving environment interacting as a functional unit” (Millennium Ecosystem Assessment, 2005). Agroecosystems are those that have been modified by people for the production of food, fuel, fiber and other products for human consumption or processing (Altieri, 1995). Characteristics of agroecosystems that are different from natural ecosystems are the use of additional energy inputs (e.g. fuel, human labor, synthetic chemicals) rather than only naturally-occurring energy sources, reduced species diversity, plants and animals under artificial rather than natural selection, and external rather than internal regulation of ecosystem processes (Pedigo and Buntin, 1994). Agroecosystems contain fewer species, greater amount of empty niche space, and lower within-species diversity (age, size, genetic diversity) within any niche (Cox, 1984). Agroecosystems must rely on external inputs (labor, nutrients, pesticides, etc.) to maintain system productivity because matter and energy is exported from the ecosystem (Pedigo and Buntin, 1994) and systems are characterized by a high rate of disturbance. In contrast, natural ecosystems recycle most of the matter and energy to support ecological processes. A major focus of agroecology is to manage agroecosystems to mimic the function and structure of natural ecosystems (Altieri, 1999; Scherr and McNeely, 2008; Lovell et al., 2010; Malézieux, 2012b). In so doing, key ecological processes such as nutrient cycling, decomposition, pest-predator interactions, hydrology, energy movement, competition, symbiosis, and soil carbon sequestration can better
maintain agroecosystem productivity and reduce external inputs (Xu and Mage, 2001; Scherr and McNeely, 2008; Thrall et al., 2011; Nicholls et al., 2017).

B. Principles of Agroecology

Agroecology uses ecological principles as the basis for management and design of farm systems, which vary with respect to socio-economic and biophysical characteristics. Therefore, the set of practices that allow external inputs to be replaced by natural processes will also vary with the particular farm context (Altieri, 1995; Gliessman et al., 1998). Nicholls et al (2016) articulate these principles, adapted from earlier work by Altieri (1995) and Reijntjes (1992), as summarized below in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Agroecological principles for the design of biodiverse, energy efficient, resource-conserving and resilient farming systems.</th>
</tr>
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<tbody>
<tr>
<td>1. Enhance the recycling of biomass, with a view to optimizing organic matter decomposition and nutrient cycling over time.</td>
</tr>
<tr>
<td>2. Strengthen the “immune system” of agricultural systems through enhancement of functional biodiversity – natural enemies, antagonists, etc., by creating appropriate habitats.</td>
</tr>
<tr>
<td>3. Provide the most favorable soil conditions for plant growth, particularly by managing organic matter and by enhancing soil biological activity.</td>
</tr>
<tr>
<td>4. Minimize losses of energy, water, nutrients, and genetic resources by enhancing conservation and regeneration of soil and water resources and agrobiodiversity.</td>
</tr>
<tr>
<td>5. Diversify species and genetic resources in the agroecosystem over time and space at the field and landscape level.</td>
</tr>
<tr>
<td>6. Enhance beneficial biological interactions and synergies among the components of agrobiodiversity, thereby promoting key ecological processes and services.</td>
</tr>
</tbody>
</table>
Agroecology uses these principles to inform particular combinations of locally adapted practices that will enable external inputs to be replaced by services provided by natural processes (Wezel et al., 2014; Nicholls et al., 2016). Ecological processes and the services they provide are inextricably linked with biodiversity; ecological processes maintain biodiversity (Noss, 1990) and biodiversity regulates ecological processes (Mace et al., 2012). As biodiversity increases, so does internal regulation of the ecosystem and provision of ecosystem services (Altieri, 1999; Garibaldi et al., 2017). Therefore, practices that sufficiently enhance biodiversity require fewer external inputs and result in economically and environmentally sustainable agroecosystems.

C. Biodiversity and Ecosystem Services

Numerous definitions of biodiversity exist, though none comprehensively and simply captures all aspects (Noss, 1990; Mace et al., 2012). Despite flaws, Mace et al. (2012) suggest the Parties to the Convention on Biological Diversity (CBD) definition which defines biodiversity as, “the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems”. Attributes of biodiversity that are particularly important for the provision of ecosystem services are species abundance, species richness, species size/weight, community/habitat area, and community/habitat structure (Harrison et al., 2014). Biodiversity determines the functioning of ecosystem processes which determine the quantity and types of services performed as well as system resilience (Chapin et al., 2000). As biodiversity increases, so does internal regulation of the agroecosystem and
provision of ecosystem services resulting in a productive system that requires fewer external inputs (Altieri, 1999).

Ecosystem services are the benefits humans derive from ecosystems as a result of natural processes (Millennium Ecosystem Assessment, 2005). The Millennium Ecosystem Assessment (MEA) organized services into four categories; provisioning (e.g. food), regulating (e.g. biological pest control), cultural (e.g. recreation), and supporting services (e.g. genetic resources for crop improvement, soil formation) (Millennium Ecosystem Assessment, 2005). While this grouping is commonly used, subsequent work led to revisions that distinguish final ecosystem services that provide goods (e.g. water regulation) from the ecological processes that underlie them (e.g. soil formation). This distinction enables economic valuation of services that can then be used to inform decisions based on cost-benefit analysis (Fisher and Kerry Turner, 2008; Fisher et al., 2008; Mace et al., 2012). As the authors indicate, this distinction is especially important for policy-makers. For decisions at the farm and field scale, ecosystem services can be sufficiently characterized as the benefits obtained from ecological processes. It should be noted that biodiversity and associated management at the landscape scale is essential to optimize provision of ecosystem services to the crop system, especially those services provided by mobile organisms such as pollination and pest predation (Tilman et al., 2002; Tscharntke et al., 2005; Altieri and Nicholls, 2012; Malézieux, 2012a; Urrutia-Escobar and Armbrecht, 2013; Cong et al., 2016).

Other examples of services to agroecosystems from ecological processes include water purification, erosion control, mineralization of nutrients, and contributions to soil structure from microorganisms (Altieri 1999; Doran and Zeiss 2000; Garbach et al. 2014;
Zhang et al. 2007 and references therein). In many cases, organisms serve multiple functions. For instance, some of the beneficial activities of soil biota are residue decomposition, increase in plant available nutrients, biological nitrogen fixation, plant growth promotion, biological control of weeds and soil pests, and improved drought tolerance (Altieri, 1999). Biodiversity underpins ecological processes (Harrison et al., 2014), performs ecosystem services (Altieri, 1999), and is also a final ecosystem service and a good (Mace et al., 2012). Consequently, the measure of biodiversity is a key indicator of ecosystem health (Rocchini et al., 2017). Conventional agriculture practices such as tillage, extensive pesticide and fertilizer applications, and monoculture result in a loss of biodiversity (McLaughlin and Mineau, 1995; Dale and Polasky, 2007). This reduces internal regulation of the agroecosystem and increases the need for external inputs. While agroecology emphasizes that the set of practices that allow external inputs to be replaced by ecosystem services are site-specific, there are practices known to facilitate provision of ecosystem services.

D. Agroecological Practices

Most of the evaluation and successful implementation of agroecological practices has been done in the tropics, sub-tropics, and developing countries (Altieri and Toledo 2011; Deguine et al. 2015; Gkisakis et al. 2016; Izquierdo et al. 2003; Santiago et al. 2012; Urrutia-Escobar and Armbrecht 2013; Wezel et al. 2014 among others). Wezel et al. (2014) reviewed agroecological practices for temperate regions, which is particularly relevant to United States crop production. They classified agroecological practices according to the three stages of transition to sustainable agriculture proposed by Hill and MacRae (1996): efficiency increase, substitution, and redesign. Efficiency-increase
practices promote a more efficient use of resources and consequently reduce inputs, substitution involves substitution of an input, and redesign refers to changes at the cropping or farm system level (Wezel et al., 2014).

Key components of agroecosystem management are crop choice (including spatial and temporal distribution), crop irrigation, and pest and disease management. Examples of agroecological practices that result in efficiency increase and substitution are crop/cultivar choice based on location, crops that promote beneficial soil microorganisms, following a nitrogen or water-intensive crop with a less demanding crop, split fertilization, using biofertilizer or organic fertilizer, and the use of natural pesticides and biological control (Wezel et al., 2014). Examples of redesign practices are crop rotations, intercropping, using allelopathic plants for weed control, direct seeding into residue, and reduced tillage (Wezel et al., 2014). There is no general recipe of practices that allow ecosystem services to replace external inputs (Altieri, 1999). Nonetheless, these examples among others can be applied in context to create an economically and environmentally sustainable crop production system.

Missing in the discussion of using ecosystem services in place of inputs is the role that viruses may have. Viruses have traditionally been considered only in a pathogenic role. Recent scientific advances in the tools and methods required to study such small entities are creating a broader understanding of virus ecology, as well as viral impact on individuals. Advances in metagenomics, where samples are taken directly from the environment, sequenced, and identified without knowing what might be found, is leading to the discovery of many, novel viruses (Malmstrom et al., 2011). If we are to capitalize on ecosystem services provided by virus activity, we must recognize viruses as
contributing more than crop disease and yield loss. Virus ecology is in its infancy, but research shows viruses are key members of ecosystems and, as such, have important functions though what these are is far from exposed. Examining the ecological roles of viruses allows identification of services provided by viruses in general, those provided to plant hosts of interest, and ways this knowledge can be applied to enhance crop productivity.

III. Virus Ecology

A. Overview

Viruses are the most abundant life forms on earth, whose presence has been found wherever other organisms exist. It is possible to find over a million different viral genotypes in 1 kg of ocean sediment (Breitbart et al., 2002), and host invasion occurs at boiling hot, acidic environments of geysers (Nuss, 1992a), extreme cold of Antarctica (Hopkins et al., 2014), driest deserts (Rohwer et al., 2009), below ~5,000 m of water (Danovaro and Serresi, 2000), and within every ecosystem between. There is not a species on earth immune to viral infection. The incredible diversity of viruses makes organizing them in neat groups across species based on activity or function challenging. This is possibly one reason virus ecology is not as developed as its counterparts. Another more prominent reason is that few were looking at viruses in this way. *Tobacco mosaic virus* was the first virus identified in 1898 (Beijerinck, 1898), and marks the beginning of researching viruses through the lens of epidemiology.

A virus particle (virion) is made up of nucleic acid and numerous copies of one or more proteins. The nucleic acid genome is either RNA or DNA, single stranded (ss) or double-stranded (ds). Most classified plant viruses contain RNA as their nucleic acid
While the particles of some viruses have an outer lipid layer (enveloped), others do not (non-enveloped). The proteins surrounding the nucleic acid, protecting it while outside the host cell, are called a capsid or coat and may also contain other proteins and macromolecules (Mateu 2013 and references therein). Once inside the cell, the plant virus particle disassembles and the genome exploits the host cell machinery to make new copies of itself and the viral proteins. The protein coat is assembled around the nucleic acid and a new virus particle is formed. In so doing, viruses take up space in the cell and interfere with cellular metabolism (Agrios, 2009).

B. Microbe Virus Ecology
1. In Aquatic Systems

Marine microbial research has contributed substantially to what is known of the role of viruses in a community environment. Viruses are the most numerous biological entities of the sea (Bergh et al., 1989; Suttle, 2005), and their abundance throughout the marine system is best predicted by bacterial abundance (Cochlan et al., 1993). Viruses are responsible for a significant proportion of bacterial death, typically a similar proportion as their other primary predator, protists (Fuhrman and Noble, 1995). Viruses are responsible for up to 100% of bacterial mortality where protists do not thrive (Fuhrman, 1999). As a result, viruses are significant drivers of the marine and global carbon cycle (Suttle, 2005, 2007).

The three basic types of bacterial virus (phage) reproduction are lytic infection, chronic infection, and lysogeny (Fuhrman, 1999). In the case of lytic infection, a virus injects its nucleic acid into the host cell directing the cell to make many more virus
particles, ultimately ending in cell burst (lysis), which releases the progeny into the environment. With chronic infection, virions are produced in small numbers and released non-lethally over several generations. If the infection is lysogenic, the nucleic acid becomes part of the host cell genome and reproduces with the host cell (prophage), neither reproducing progeny within the cell nor destroying the cell (Gill and Abedon, 2003). Events such as host stress can trigger lysogenic infections to become lytic (Fuhrman and Noble, 1995). It is the lysogenic interactions that are thought to protect hosts from virulent phage and provide hosts with new functions coded by the virus genome (Lenski, 1988; Fuhrman, 1999).

Through cell lysis, the virus progeny are released into the environment as well as the cellular remnants of dissolved molecules, cell fragments, and colloids (dissolved organic matter). This dissolved organic matter is food that is available to bacteria (Shibata et al., 1997; Fuhrman, 1999; Suttle, 2007). Cell lysis creates a microbial loop of dissolved organic matter from primarily bacteria, but also phytoplankton, then feeding bacteria and prohibits the organic matter and nutrients from moving up the food chain to protists and beyond. Compared to an identical food web with no viruses, this system has been shown to increase bacterial production and respiration by 33% while reducing production of organisms higher in the food chain (Fuhrman, 1999). The net effect of the availability of dissolved organic matter is an increase in marine system respiration, CO$_2$ release, and nutrient cycling contributing significantly to the carbon cycle of the ocean and the globe (Suttle, 2005, 2007; Rohwer et al., 2009).

Within bacterial and phytoplankton communities, phages maintain biodiversity by 1) limiting population sizes of the top competitors for resources, thereby allowing resources
for less competitive species and 2) influencing the composition of dissolved organic matter from lysed cells that selectively provides food for other heterotrophic species (Sandaa et al., 2009). Viruses have been shown to control population sizes well below the limitation imposed by resource availability (Lenski, 1988), and they are important agents in the control of harmful algal blooms (Sieburth et al., 1988; Bratbak et al., 1993; Milligan and Cosper, 1994; Jacobsen et al., 1996; Gobler et al., 1997; Nagasaki and Yamaguchi, 1998; Fuhrman, 1999; Brussaard, 2004). Finally, one of the most recognized ecological functions of viruses is the exchange of genetic material between hosts, kingdoms, and ecosystems (Kimura et al., 2008; Rohwer et al., 2009).

Ecological studies of viruses in marine systems provide a broader perspective of the role of viruses in ecosystems and a starting point for deciphering their role in soil microbial communities. As is the case for marine microbial communities, soil microbial communities are a model system for identifying the roles of viruses in ecosystems because of their high abundance, relative biological simplicity, and high turnover rates.

2. In Soil Systems

Viral ecology in soil systems is not as well understood as aquatic systems, but some of the major functions of viruses in aquatic environments can be attributed to those on land. For instance, phages control bacterial populations (Douglas, 1975), participate in maintaining biodiversity, provide immunity against virulent viruses, and exchange genetic material between hosts (Marsh and Wellington, 1994). Compared to the sea, there are equivalent numbers of bacteria in soil, densely populated, with a much wider range of environmental habitats for viruses. Therefore, it is likely that viruses have just
as many, though substantially different, roles in the soil as in aquatic environments (Kimura et al., 2008).

Some environmental conditions that directly affect viral activity and abundance in soils, though the effect is highly dependent on viral type, are temperature, pH, moisture content, clay-type, and salinity (Kimura et al., 2008). Adsorption of viruses to clay particles increases their survivability in soils. In general, phages are negatively charged and adsorb to particles via the clay-cation-virus bridge, with divalent cations better bridges than monovalent (Kimura et al., 2008). pH influences the charge on both viruses and clay particles (Carlson Jr et al., 1968; Lipson and Stotzky, 1983). Organic matter weakens the binding of viruses to soils (Zhuang and Jin, 2003). Along with adsorption, temperature seems to be the main factor influencing virus survivability in soils (Hurst et al., 1980; Yates et al., 1985), with, in general, lower temperatures associated with longer periods of survival, latency, and smaller burst size (Leonardopoulos et al. 1996a,b; Zachary, 1978; Straub et al., 1992; Kimura et al., 2008).

Moisture content also influences viral activity; viral inactivation rates increase as soil moisture content decreases (Yeager and O’Brien, 1979). Moisture content is a key factor in viral migration, as viruses move up and down in soils by groundwater and sediment transport through pores. Other important factors that influence migration are binding strength and its dependent factors, as well as soil structure and texture (Kapuscinski and Mitchell, 1980; Keswick and Gerba, 1980; Jin et al., 2000). The factors that affect survivability, infectivity, and abundance of phages in soil systems are different than aquatic, and some of the important ecological functions of phages may not translate to soil environments.
One example of a major role that viruses play in aquatic environments that is likely different in soils is with regard to biogeochemical nutrient cycles. Viruses contribute so much to the biogeochemical cycle in the ocean due to the high amount of bacterial biomass and fast microbial community turnover time due to lysis. In contrast, fungal biomass is typically the most abundant biomass in undisturbed soils (Anderson and Domsch, 1980) and turnover time is 1 year or more (Jenkinson et al., 1977; Whitman et al., 1998). Lysogeny is thought to be a survival strategy of viruses when host abundance is low, and lytic infection in coastal waters was found to occur only when combined virus and bacterial numbers were at least $10^{12}$ mL$^{-1}$ (Wilcox and Fuhrman, 1994). Kimura et al. (2008) point out that adsorption to soil particles will likely influence the requisite numbers associated with a change to lytic lifestyle. The value may be higher with viral adsorption to soil particles, restricting movement and decreasing likelihood of host contact, or lower when both hosts and viruses accumulate on particles (Kimura et al., 2008). The unknown extent of microbe mortality due to lysis and the role of fungal viruses (mycoviruses) in biogeochemical cycles combined with a microbial loop in which plant exudates and residues have a dominant role in cycling nutrients means that the role of viruses in this context is an open question (Kimura et al., 2008).

The role of viruses of soil microbes in agroecosystems and their potential to provide services through agroecological management is unknown. All of the above environmental conditions that affect virus survivability, infectivity, and movement in the soil can be manipulated by agriculture practices to varying degrees. Further research is needed on specific virus species of interest to agriculture production and how practices that affect environmental conditions impact the activity of these viruses in the soil. It is
possible that bacterial populations both beneficial and deleterious to agriculture can be effectively managed through promotion or inhibition of specific viral activities by manipulation of the soil environment. This important sector should not be ignored as agroecology develops.

C. Plant Virus Ecology

The tenth report from the International Committee on the Taxonomy of Viruses (ICTV) classifies approximately 1400 species of plant viruses (ICTV, 2017), mostly pathogenic viruses of crop plants. This is a considerable increase from the 900 species classified in the ninth report (King, 2011). This large increase shows the speed at which new viruses are being identified and the likelihood that many more are yet unknown. New detection technology is enabling research beyond the economic impact of known crop viral diseases to identify important ecological roles of viruses. Ecosystems lie on a continuum from wild communities to highly managed plant production systems (Alexander et al., 2014). The study of wild plant communities is essential to viral ecology studies given the greater diversity of host species (diversity in plant taxa, age structure, etc.), domination of perennial plants in nature versus annual plants in agro-ecosystems, and more complex abiotic and biotic interactions (Malmstrom et al., 2011). These factors combine to provide a broader perspective of plant-virus interactions in general that can then be applied to understanding virus activity in agroecosystems.

Viruses contribute significantly to marine biogeochemical cycles, total system respiration, and productivity of species at various trophic levels. In contrast, little to nothing is known in these regards with respect to terrestrial ecosystems, an area of research worthy of attention (Malmstrom et al., 2011). The first virus characterized was
that of a plant disease, *Tobacco mosaic virus* (Beijerinck, 1898) which set the stage for thinking of viruses strictly as disease-causing agents (Roossinck, 2010), though the majority of plant viruses do not cause disease (Roossinck, 2005). Recently, plant virus ecology has focused on the study of the roles of plant viruses and associated vectors in natural and managed ecosystems and the effect of system properties on virus and vector evolution and distribution (Malmstrom et al., 2011). Agroecology must incorporate an improved understanding of plant virus ecology in order to manage agroecosystems to deliver a full range of ecosystem services.

1. **Lifestyles of Plant Viruses**

There are four possible lifestyles of plant viruses; acute, chronic, latent (termed ‘persistent’ by Roossinck), and endogenous (Roossinck, 2010). Acute infections are those typically associated with crop disease and are characterized by cell-to-cell movement mediated by movement proteins, horizontal transmission by vectors, a tendency to accumulate to high levels in plants, and causing obvious symptoms. The infection cycle ends with either successful defense by the host, host death, or (uncommonly) a switch to chronic lifestyle (Roossinck, 2010, 2012). Chronic infections are very similar to acute, but infections last longer and may or may not have obvious symptoms. Latent plant viruses, in contrast, do not move between cells but are found in every cell, are only transmitted vertically, maintain low titer in cells, and are usually asymptomatic (Cooper and Jones, 2006; Roossinck, 2010). A characteristic common to biological systems, it is likely that viral infection types exist on a continuum from latent to acute (Roossinck, 2013). Endogenous viruses are those that incorporate into the host genome, typically thousands of years ago. Attributed roles of endogenous retroviruses
include immunity from acute viruses, provision of functional genes, and horizontal gene transfer (Roossinck 2011 and references therein). Beyond this, ecological roles of endogenous viruses will not be discussed further as they are very distinct.

2. Latent Viruses

In recent analyses of wild plant communities in diverse environments, the majority of viral infections identified were those of a latent nature (Roossinck, 2012, 2013). Roles of latent viruses, with few known phenotypes, are not clear but evidence suggests these viruses can benefit the host by providing new, functional proteins and protection from pathogenic viruses, promoting host group survival (Villarreal, 2009; Roossinck, 2010; Roossinck and J., 2011). Given their ubiquity, it also likely that latent viruses are a source of new, emerging viruses through recombination/reassortment with acute viruses (Roossinck 2010, 2013, Rastgou, 2009). Latent viruses are found in numerous crop plants including jalapeno pepper, rice, barley, bell pepper, strawberry, and melon (Roossinck 2012 and references therein) with no known function though virus free comparison plants are difficult to find. Given their longevity and avoidance of RNA silencing mechanisms, it is likely that latent viruses are mutualistic (Roossinck, 2012). A few examples of mutualistic latent viruses have been identified (see section below). The role of latent viruses in plant communities is only beginning to be understood, and continued research is likely to elucidate relationships of latent viruses and plant hosts. For a thorough review of latent viruses, including role of fungi and associated latent viruses in virus evolution, see reviews by Roossinck (Roossinck 2010, 2011, 2012, 2015).
3. Acute Viruses

The death and disease we associate with viruses in general is the result of acute viruses, though these viruses in wild plants are often asymptomatic and there is no correlation in wild plant communities between presence of symptoms and viral infection (Roossinck, 2012). As discussed below, some acute viruses have known benefits under certain environmental conditions (Xu et al., 2008; Roossinck, 2011). As with other pathogens, acute viruses flourish in monoculture (Alexander et al. 2014; Roossinck 2013), though plants surveyed in wild communities often have multiple infections consisting of both latent and acute viruses (Roossinck and García-Arenal, 2015). Acute virus ecology is particularly relevant to crop production given horizontal transmission by vectors within and between ecosystems and the association of acute viruses with disease. Interactions among viruses, vectors, plants, and the environment are complex and intertwined, though some delineation is attempted here. For the rest of this section, ‘virus’ refers to acute plant virus.

4. Viruses Affect Community Composition

Plant viruses range from being able to infect only a single species to hundreds of species, but spread is limited by a limited range of vectors. Consequently, vector host preference and movement are major determinants of virus spread (Power, 2008). Most viruses are naturally transmitted by insect vectors, especially whiteflies (Aleyrodidae), thrips (Thysanoptera), and aphids (Aphidoidea), though mites, nematodes, fungi, and dodder are also carriers (Agrios, 2009). Several controlled studies have demonstrated the negative impact of common crop viruses on wild plant species’ populations grown in unnatural settings (Funayama et al., 1997; Power and Mitchell, 2004; Navas et al., 2009;
Vincent et al., 2014). To what extent the reduction in population fitness translates to natural environments is unclear, given more complex biotic and abiotic interactions in natural systems. For instance, the effects of natural predators on insect vector populations and the ability of healthy plants to compensate for the loss of productivity of infected individuals over time is likely to influence disease impact on natural communities (Navas et al., 2009). These studies highlight the potential of virus infection to affect genotypic diversity in wild plant populations, given more resistant genotypes will produce more offspring.

The few studies that have been conducted on the effects of viruses on plant community composition in natural settings indicate viruses can have a significant role in the invasion of introduced plants on existing plant communities. These involve barley and cereal yellow dwarf viruses (BYDV, CYDV), which are ssRNA luteoviruses transmitted to numerous wild and crop grass species by several aphid species (Miller and Rasochová, 1997; Power, 2008). In one study, invasion of the winter annual weed African wiregrass [Ventenata dubia (Leers) Coss.] infected with BYDV facilitated infection of native grass species on prairie lands of Idaho and Washington (Ingwell and Bosque-Pérez, 2015). This is the first report of BYDV infection in the region, which may have consequences for conservation of native grassland species and provides a new source of infection for Poaceae crops (Ingwell and Bosque-Pérez, 2015). In other studies on California grasslands, research by Malmstrom et al (2005) demonstrated that invasive species draw aphid populations to areas that then spread BYDV/CYDV to native populations. This causes native population decline and the spread of invasive species that are otherwise inferior competitors (Malmstrom et al., 2005a; b). Roossinck suggests
invasive species may carry beneficial viruses that may help them compete with native populations (Roossinck, 2013).

5. Viruses Affect Vector Behavior

Numerous examples have been found in which viruses influence vector behavior. In one study, aphids infected with BYDV preferred non-infected wheat plants, while uninfected aphids preferred infected plants (Ingwell et al., 2012), which would encourage transmission rates in plant populations. In another, squash plants (Cucurbita pepo L. cv. Dixie) infected with Cucumber mosaic virus (CMV) emitted volatiles similar to those released by healthy plants. The volatiles attracted aphids to the diseased plants, though aphids did poorly on the diseased plants and quickly moved off (Mauck et al., 2010). Given that vectors acquire CMV from the host quickly, transmission of CMV in plant populations is increased by quick dispersal. Transmission mechanisms of a virus appear to influence the host quality for vectors (Mauck et al., 2012; Roossinck, 2013). Virus infection of plants can alter vector behavior, population growth, and reproduction (Malmstrom et al., 2011 and references therein).

6. Biodiversity

Especially relevant to crop production systems is how ecosystem properties affect virus emergence, transmission, and pathogenicity. In research plots, the infection rates of viruses increased with increasing levels of phosphorus (Borer et al., 2010) and this increase affected different viral strains to varying degrees (Seabloom et al., 2013). Ecosystem biodiversity influences virus distribution and evolution. In one of the earliest examples, beet curly top disease outbreaks in Southwestern sugar beet fields were found to be the result of the sugar-beet leafhopper vector traveling long distances to find new
hosts when weed hosts senesced (Carter, 1930). Populations of leafhopper increased substantially in the desert location due to proliferation of annual weeds caused by diversity loss of the rangeland (Piemeisel et al 1951, Malmstrom et al. 2011). As the weeds senesced, large populations of leafhopper migrated hundreds of miles to the sugar beet fields.

The effect of biodiversity on viral disease is demonstrated by studies of the wild pepper, Chiltepen [Capsicum annuum L. var. glabriusculum (Dunal) Heiser & Pickersgill], in Mexico. Chiltepen is a popular food source for which management practices range from cultivated to wild stands. Infections by Pepper golden mosaic virus and Pepper huasteco yellow vein virus were positively correlated with management intensity (ecosystem simplification) and heterogeneity of pepper genotypes decreased with cultivation (Pagán et al., 2012; Roossinck and García-Arenal, 2015). Infected wild plants also have lower disease levels than infected, cultivated peppers (Rodelo-Urrego et al., 2013). In general, loss of ecosystem biodiversity is associated with an increased incidence of virus disease (Roossinck and García-Arenal 2015 and references therein). Crop production systems based on agroecological principles are likely to have fewer virus epidemics compared to conventional, monoculture systems.

7. Considerations

Plant virus ecology is a quickly expanding field and only a brief overview was provided here. Nonetheless, it is clear that the traditional view of viruses as pathogens is incomplete, though the spread of viral disease is an important component of agroecology. For instance, if biodiversity reduces epidemic frequency, how should wild and weedy hosts of crop viruses be best managed to prevent crop disease and how can we best
increase agroecosystem diversity (prairie strips, intercropping, etc.) without providing alternate plant hosts for viral pathogens (Alexander et al., 2014)? Other important ecological questions include the relationship between host biodiversity and virus biodiversity, the rate and influence of co-infections in natural and managed communities, and the prevalence of asymptomatic or beneficial viruses in ecosystems (Wren et al., 2006). The following review of viruses beneficial to their plant hosts demonstrates that viruses contribute more to plants and ecosystems than disease.

IV. Viruses Beneficial to their Plant Hosts

This review section takes an ecological approach and focuses on naturally occurring plant viruses that have been identified to have a beneficial effect on their plant hosts, with implications for the importance of maintaining agroecosystem biodiversity. Viruses also infect many economically important crop pathogens which is also beneficial to crop production systems. They are known to attenuate virulence of fungal pathogens (hypovirulence) (Anagnostakis, 1982; Buck, 1988; Nuss, 1992b; Ghabrial and Suzuki, 2009), and kill insect (Lacey et al., 2001, 2015) and bacterial pathogens (Gill and Abedon, 2003; Tobias Abedon et al., 2017), which are important roles of viruses in the agro-ecosystem, but are not the subject of this review. For a review of these functions, see cited references.

A. Cross Protection

Cross protection is a well-known example of the ways in which viruses have benefited crop production throughout history. First described by McKinney (1929), cross protection is the phenomenon in which infection of a plant by a mild strain of a virus
produces tolerance of or resistance to a more virulent strain. In this first experiment, leaf-yellowing symptoms of the pathogenic, “light green strain” of *Tobacco mosaic virus* (TMV: Genus *Tobamovirus*) were reduced after inoculation with TMV “yellow mosaic strain”. This was followed in 1933 by an experiment that showed infection by a non-pathogenic strain of *Potato virus X* (PVX: Genus *Potexvirus*) prevented infection by a pathogenic strain of PVX (Salaman, 1933a). Since then, many field and lab experiments have confirmed the phenomena across multiple plant and virus families, the majority are listed in Table 2 prepared by Shiboleth and Gal-On (Shiboleth and Gal-On, 2006).

**Table 2. Cross-protection experiments effective in the field or laboratory**

<table>
<thead>
<tr>
<th>Protecting virus</th>
<th>Challenging virus</th>
<th>Host plant</th>
<th>Test site</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfamovirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alfalfa mosaic virus mild strain</td>
<td>AIMV Severe strain</td>
<td>Bean</td>
<td>lab</td>
<td>(Hull and Plaskitt, 1970)</td>
</tr>
<tr>
<td>Badnavirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocoa swollen shoot virus</td>
<td>CSSV wild type</td>
<td>Cocoa</td>
<td>field</td>
<td>(Hughes and Ollenu, 1994)</td>
</tr>
<tr>
<td>Caulimovirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cauliflower mosaic virus UN130 strain</td>
<td>CaMV Cab S strain</td>
<td>Turnip, Brussels sprout</td>
<td>lab</td>
<td>(Tomlinson and Shephard, 1978; Zhang and Melcher, 1989)</td>
</tr>
<tr>
<td>Closterovirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrus tristeza virus mild strains</td>
<td>CTV severe strain</td>
<td>Citrus</td>
<td>field</td>
<td>(Costa, A. S.; Muller, 1980)</td>
</tr>
<tr>
<td>Cucumovirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cucumber mosaic virus (S) mild strain</td>
<td>CMV(P) severe strain</td>
<td>Tomato, Tobacco Squash</td>
<td>lab</td>
<td>(Dodds, 1982; Dodds et al., 1985)</td>
</tr>
<tr>
<td>CMV with satellite</td>
<td></td>
<td>Pepper, Melon</td>
<td>lab, field</td>
<td>(Yoshida et al., 1985; Montasser et al., 1998)</td>
</tr>
<tr>
<td>Tomato aspermy virus</td>
<td>virulent TAV strains</td>
<td>Tomato</td>
<td>lab</td>
<td>(Kuti and Moline, 1986)</td>
</tr>
<tr>
<td>Furovirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beet soilborne mosaic virus</td>
<td>Beet necrotic yellow vein virus</td>
<td>Sugarbeet</td>
<td>lab</td>
<td>(Mahmood and Rush, 1999)</td>
</tr>
<tr>
<td>Geminivirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African cassava mosaic virus-Uganda</td>
<td>Virulent ACMV strains</td>
<td>Cassava</td>
<td>field</td>
<td>(Owor et al., 2004)</td>
</tr>
<tr>
<td>Ilarvirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple mosaic virus</td>
<td>Virulent ApMV strains</td>
<td>Apple</td>
<td>field</td>
<td>(Chamberlain et al., 1964)</td>
</tr>
<tr>
<td>Luteovirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barley yellow dwarf virus-MAV</td>
<td>BYDV PAV</td>
<td>Cereal, Oat</td>
<td>lab</td>
<td>(Jedlinski and Brown, 1965; Wen et al., 1991)</td>
</tr>
<tr>
<td>Potato leaf roll virus mild strain</td>
<td>Severe strain of PLRV</td>
<td>Potato</td>
<td>lab</td>
<td>(Webb et al., 1951; Harrison, 1958)</td>
</tr>
<tr>
<td>Nepovirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabis mosaic virus</td>
<td>Grapevine fanleaf virus</td>
<td>C. quinoa</td>
<td>lab</td>
<td>(Huss et al., 1989)</td>
</tr>
<tr>
<td>Tomato ringspot virus</td>
<td>virulent ToRSV strains</td>
<td>Peach</td>
<td>lab</td>
<td>(Bitterlin and Gonsalves, 1988)</td>
</tr>
</tbody>
</table>
The most successful commercial applications have been in the control of the devastating *Citrus tristeza virus* (CTV) in citrus orchards in many regions of the world where citrus are grown, such as Brazil, Australia, Japan, and the United States (Costa and Muller, 1980; Shiboleth and Gal-On, 2006). *Papaya ringspot virus* (PSRV) is the most widespread and destructive papaya virus (Gonsalves, 1998). Control has been successfully achieved in many tropical regions, though the avirulent mutant was achieved in the laboratory through nitrous acid mutagenesis and was not found to be naturally occurring (Gonsalves, 1998). In perhaps one of the final examples of the current,
commercial use of cross protection, the cucurbit pathogen *Zucchini yellow mosaic virus* (ZYMV) is effectively controlled in Israel through inoculation with a naturally occurring mild strain (Desbiez and Lecoq, 1997, Yarden et al, 2001).

Identified mechanisms responsible for cross protection are coat-protein (CP) mediated resistance and RNA silencing (Shibolet and Gal-On, 2006; Natsuaki, 2014), with most examples based on RNA silencing. In the case of CP-mediated resistance, infection by the mild strain prevents protein uncoating of the virulent strain (Beachy, 1999). This prevents any subsequent translation and replication (Shibolet and Gal-On, 2006). CP-mediated resistance is not the only possible mode of action, given CP-defective viruses and viroids have also been shown to protect against pathogenic strains (Shibolet and Gal-On, 2006). RNA silencing is used by hosts to defend against viruses and related entities, known as post-transcriptional gene silencing (PTGS) in plants. The dsRNA produced during viral replication triggers the plant to degrade viral RNA via a nuclease silencing complex termed the RNA-induced silencing complex (RISC). RISC can move from infected to non-infected cells via plasmodesmata, priming them to defend against virus entry. Co-evolution of viruses and plants has resulted in viruses coding for proteins that suppress the RNA silencing-based defense mechanisms used by plant hosts (Roth et al., 2004). RNA-silencing-mediated cross protection occurs, through a chain of events, as infection by the first, mild strain engages host silencing mechanisms and primes them for degradation of the more virulent strain (Shibolet and Gal-On, 2006).

Despite widespread evidence of its effectiveness, cross protection has not been developed more commercially for a number of important reasons. These include the cost and difficulty of inoculation, incomplete protection in some cases, risk of mutating to
become more severe, and development of technologies such as transgenic crops that integrate parts of the virus genome into the plant (Shiboleth and Gal-On, 2006). Nonetheless, cross protection is an important example of a benefit viruses may provide to crop production from an ecological perspective. Most plants are infected by multiple viruses and greater biodiversity is correlated with a reduction in disease prevalence. As it now seems likely that acute viruses from the field recombine with latent viruses of surrounding vegetation producing attenuated viral strains (Roossinck and García-Arenal, 2015), cross protection interactions may be taking place regularly in crop fields depending on the extent of biodiversity within and around the agroecosystem.

B. Beneficial plant-virus interactions in response to biotic and abiotic stress

Viruses are obligate pathogens, meaning they live in ‘intimate association with dissimilar entities’ (Roossinck, 2011) and this association is required for replication and survival of the virus. It is becoming evident that the nature of the virus/host relationship, which runs along a continuum from antagonistic to mutualistic, is dependent on the environment. A virus that is antagonistic under normal environmental conditions can become beneficial to the host under stressful conditions (Bao and Roossinck, 2013). The majority of beneficial interactions listed here exemplify this finding. As in the paper by Bao and Roossinck (2013) and based on the definition of mutualistic symbiosis (Holland et al., 2002), viruses here are defined as beneficial when the result of the interaction is positive (the plant performs better) compared with non-infected plants. The identification and study of viruses that benefit their plants hosts underlines the importance of viruses as contributing members of robust ecosystems.
1. Viruses Alter Outcome of Biotic Interactions

   a) Fungal gnats are less attracted to white clover infected by White clover mosaic virus

   There have been many studies evaluating the effect of plant viruses on vectors, typically showing that many plant viral diseases make plants more attractive to their vectors (Casteel et al., 2014). van Molken et al (2012) examined the effect of a virus, White clover mosaic virus (WC1MV), on the behavior of a non-vectoring herbivore, fungal gnats (Bradysia sp.; Sciaridae), during infection of Trifolium repens L. (white clover) (Van Mölken et al., 2012). The study evaluated whether infection by WC1MV had any impact on the effects of fungus gnat feeding on plant growth, attraction of adult female gnats to the plants, and emission of volatile blends by white clover plants (Van Mölken et al., 2012). WC1MV is mechanically spread, rather than transmitted by insect vectors (Tapio, 1970).

   To conduct the study, van Molken et al. used white clover plants of the same genotype (A120) grown from cuttings planted five years prior and grown in a greenhouse. Number of ramets and total plant biomass were used as measures of plant fitness. Fungus gnats were collected from the green house, allowed to propagate by laying eggs into potting soil, and gnats never previously exposed to plant tissue were used for the experiments. Plants were either mechanically inoculated with WC1MV or mock inoculated using only buffer.

   Results indicated that fungal gnat feeding had a greater negative impact on plant growth than WC1MV infection. Fungal gnat infestation reduced plant biomass by 52%, whereas WCM1V infestation reduced it by only 27%. Total number of ramets and percentage of branches on the main stolon were significantly reduced by fungal gnat
feeding, while WC1MV infection did not affect these (Van Mölken et al., 2012). Most relevant, virus infection strongly impacted the attractiveness of adult females to white clover plants; females preferred non-infected plants (67%) over WC1MV infected plants (33%) when given a choice between the two. Volatile blends emitted by control and infected blends were significantly different, and further analysis indicated that only two compounds were significantly responsible for this difference between the two groups; β-caryophyllene and benzonitril, especially β-caryophyllene which was not even detected in the canopy of virus-free controls (Van Mölken et al., 2012).

b) Zucchini yellow mosaic virus (ZYMV) reduces transmission of bacterial wilt in wild gourd populations

Wild gourd (Cucurbita pepo ssp. texana) is frequently infected in wild populations by ZYMV and/or the causal agent of bacterial wilt, Erwinia tracheiphila (Shapiro et al., 2013). Erwinia tracheiphila is vectored by striped cucumber beetles (Acalymma vittatum) (Garcia-Salazar et al., 2000). This study was based on previous research that indicates ZYMV-infected plants contract the fatal bacterial wilt disease at reduced rates compared to non-ZYMV-infected gourds and that co-infections are significantly fewer than chance predicts (Sasu et al., 2009, 2010). In previous work, it was found that ZYMV infected plants produce fewer flowers than non-infected plants and that the flowers that are produced emit less floral odors (Sasu et al., 2009, 2010). Floral odors are partially responsible for attracting the beetle to the gourd because beetles congregate to feed and mate on the flowers (Lewis, 1990, Shapiro et al. 2012). A reduction in the number of flowers produced and attenuation of floral odors is likely a factor affecting the prevalence of co-infection (less than expected by chance) of the two pathogens in wild gourd (Shapiro et al. 2012). This study was aimed at investigating
whether ZYMV infection also induced systemic acquired resistance (SAR) in the plant making it less susceptible to bacterial wilt infection (Shapiro et al., 2013).

To measure induced levels of phytohormones related to plant defense, wild gourd seeds (C. pepo spp. texana) collected from wild plant populations in Texas and grown at the Pennsylvania State University research farm were germinated, transplanted to pots, and grown in chambers. The plants were divided into three treatment groups: ZYMV inoculated, mock inoculated, and untouched. Upon development of ZYMV symptoms (7 days after inoculation), half of the plants in each of the mock-inoculated and ZYMV-infected treatment groups were inoculated with bacterial wilt and the other half mock-inoculated with water. Forty-eight hours later, plant tissue was collected and concentrations of jasmonic acid and salicylic acid were determined.

To determine whether infections by ZYMV or bacterial wilt-causing Erwinia tracheiphila influenced induced responses of gourd plants to beetle-feeding, seedlings were inoculated with Erwinia tracheiphila, ZYMV, or mock inoculated. When all of the truly inoculated plants showed symptoms of disease, half of the plants from each of the three treatment groups had one beetle confined to the youngest expanded leaf for 15 hours. A small portion of leaf tissue was subjected to phytohormone analysis according to the procedure described in the previous experiment. Finally, to identify any differences between healthy and ZYMV-infected plants to bacterial wilt susceptibility, seedlings were ZYMV-inoculated or mock inoculated, allowed to grow for 7 days, and then challenged with bacterial wilt. This experiment was done twice; once with Erwinia tracheiphila concentrations similar to what would be found in the field and once with
higher inoculation levels. Data was collected on the length of time to first appearance of wilt disease symptoms and disease progression over time.

In the first described experiment, inoculation or mock inoculation with ZYMV and/or *Erwinia tracheiphila* without beetle feeding, ZYMV-infected leaves had higher levels of SA, both before and after infection by *Erwinia tracheiphila*, compared to plants uninfected by either pathogen. Co-infection of *Erwinia tracheiphila* and ZYMV also resulted in higher SA levels in leaves, though *Erwinia tracheiphila* infection alone did not result in higher SA. JA was not detected in the pathogen treatments. In the second experiment, beetle feeding did not influence levels of SA in any of the treatment groups though higher SA levels were again found in ZYMV-infected plants compared to healthy plants or plants infected only by *Erwinia tracheiphila*. Significant JA response was induced by beetle feeding across all treatments, particularly high in plants infected by *Erwinia tracheiphila* compared to uninfected or ZYMV-infected gourds.

In the last described experiment, with lower levels of *Erwinia tracheiphila* inoculum, there was no significant difference in the percentage of plants contracting wilt disease or the progression of wilt infection between healthy or ZYMV plants. Using higher concentrations of *Erwinia tracheiphila* inoculum, wilt spread to the second leaf slightly slower in ZYMV plants than uninfected controls and whole-plant wilting also occurred later (Shapiro et al., 2013). The study demonstrates that while infection with ZYMV induces SA response that may slightly reduce infection by *Erwinia tracheiphila*, the majority of observed reduction of wilt disease prevalence in ZYMV-infected wild gourds can be attributed to previous findings that ZYMV reduces flower number (Sasu et al., 2009, 2010) and flower odors that attract wilt-vectoring beetles to the plant (Shapiro
et al., 2012), resulting in less contact between ZYMV-infected gourds and vectors of wilt disease.

Though ZYMV is pathogenic, infection results in reduced transmission of wilt bacteria due to reduced volatile compound emissions that attract the *Erwinia tracheiphila* vector, the striped cucumber beetle (Shapiro et al., 2012). This meets our definition of beneficial, given that wilt disease and ZYMV are ubiquitous in natural populations of wild gourd and that wilt invariably causes death of the host within days while wild gourd plants can potentially recover from ZYMV infection. Even in this experiment, two plants had to be subsequently excluded from the study due to recovery of the plants from ZYMV infection.

c) *Virus infection makes plants more attractive to bumblebee pollinators*

Tomato (*Solanum lycopersicum* L. var. lycopersicum) and *Arabidopsis thaliana* plants infected with *Cucumber mosaic virus* (CMV) were evaluated for attractiveness to non-vectoring bumblebee (*Bombus terrestris*) pollinators. Volatile organic compounds known to enhance plant attractiveness to bees were analyzed in CMV-infected tomato plants. Bee pollination enhances seed and fruit production and spreads pollen to other plants (Groen et al., 2016 and references therein).

“Flight arenas” were set up containing ten tomato plants, five CMV-infected and five non-infected. Cups containing 30% sucralose solution were placed on top of towers allowing odors to escape but preventing the bees from touching or seeing the plants. Bees preferred the CMV-infected towers hiding tomato plants over mock-inoculated towers (Groen et al., 2016) and analysis of volatile organic compounds in the headspace confirmed blend differences between the two treatments.
**d) Viral coat protein gene suppresses nodulation of legume, Lotus japonicus**

In this case, it is a gene of a latent virus identified as responsible for having a potential beneficial effect in its plant hosts. Rhizobia bacteria and legume hosts form a mutualistic symbiotic relationship in which Rhizobia infect nodules on legume plant roots and convert atmospheric nitrogen to plant-available nitrogen compounds in exchange for photosynthates. While this interaction has the potential to benefit both parties, excessive nodulation has been shown to negatively impact plant growth (Nishimura et al., 2002).

The coat protein of *White clover cryptic virus 1* (WCCV1) is encoded by the gene *Trifolium repens* early nodulin downregulation 1 (*TrEnodDR1*) (Boccardo and Candresse, 2005). *TrEnodDR1* was found to be down-regulated during nodule formation of white clover (*T. repens* L.) induced by presence of *Rhizobium leguminosarum* bv. *trifolii* (Suzuki et al., 2001). This led to the hypothesis that artificial expression of the coat protein gene (*TrEnodDR1*) of WCCV1 would impact root nodulation (Nakatsuksa-Akune et al., 2005). To conduct the study, model legume *Lotus japonicus* was transformed using Agrobacterium-mediated transformation. Plasmid containing the coding region for green fluorescent protein (sGFP) and *TrEnodDR1* was inserted in some treatment plants, as well as an empty vector with sGFP to generate control plants for comparison. sGFP is used to determine if the transformation was successful. Hairy roots were then inoculated with *Mesorhizobium loti* MAFF303099.

Plants expressing *TrEnodDR1* had lower total root length and fewer lateral roots than controls. The number of nodules per unit of root length was also reduced. Shoots of transformed whole plants were significantly shorter. The number of nodules per plant
was greatly reduced in *TrEnodDRI* plants compared to control. The phytohormone abscisic acid (ABA) is an important regulator of plant growth, development, and response to external stress (Nakatsukasa-Akune et al. 2005 and references therein) and has been shown to influence the number of root nodules formed (Suzuki et al., 2004). ABA concentrations in roots were increased in *TrEnodDRI* plants. Abamine is known to interfere with and suppress ABA production in plants. Treatment of roots with abamine decreased ABA levels and total root length, number of nodules, and number of lateral roots of *TrEnodDRI* plants reached those of control plants after abamine treatment. In addition to increased ABA concentration, expression of a number of plant defense response genes were also enhanced. The authors concluded that *TrEnodDRI* suppresses nodulation of plant roots via an increase in ABA concentrations in root tissues, possibly as a result of activating the immune response of the plant (Nakatsukasa-Akune et al., 2005).

In environments where sufficient soil nitrogen is present, the use of the virus coat protein gene by plants to suppress nodulation can be beneficial. Reduced nodulation can facilitate enhanced plant growth due to conservation of photosynthates for plant use rather than bacterial consumption. This study provides an exciting example of an infection by a latent virus influencing plant host physiology. Further research is needed on the effect of latent plant virus infections on development, growth, and response of hosts to various environmental conditions.
2. Viruses impact host performance in response to abiotic stress

The primary challenge facing agriculture today is how to produce more food with fewer resources. Our resource base is finite and ever decreasing, global population is increasing, and changes in climate are expected to further strain available resources and alter crop production patterns throughout the world (Food and Agricultural Organization of the United Nations, 2017). Millions of dollars each year are spent on agriculture research programs focused on increasing productivity of crop plants and decreasing system inputs. Examples of such programs include water-use efficiency, salt tolerance, nitrogen-use efficiency, and improved set in high or low temperatures. Some viruses have the potential to beneficially influence host response to abiotic stress. These interactions demonstrate the roles of viruses in ecosystems other than as pathogens and provide insight into mechanisms responsible for improved stress tolerance.

a) Plant-fungal-virus tritrophic symbiosis confers heat tolerance

Though this review is focused on beneficial plant viruses, the following case of a fungal virus that benefits its fungal host and the plant host of the fungus is remarkable. In Yellowstone National Park, a panic grass (*Dichanthelium lanuginosum*) is found growing in geothermal soils reaching an excess of 50 °C. The plant is infected by the endophytic fungus, *Curvularia protuberata*. The fungus was at one point thought to be solely responsible for conferring heat tolerance to the plants that could otherwise not survive the high temperatures (Redman et al., 2002). Subsequent studies have shown that a virus, *Curvularia thermal-tolerance virus* (CThTV), is also required for survival of both the fungus and the plant. Panic grass requires infection by the fungus, and the fungus
requires infection by the virus to survive the extreme heat; all three partners are required for thermal tolerance (Márquez et al., 2007).

To demonstrate this, Marquez et al. used wild-type fungus isolates determined to have high titers of CThTV (given high concentration of dsRNA which does not normally occur in virus-free fungal cells) and identical isolates cured of viral infection. Panic grass plants infected by CThTV-infected fungi were able to survive soil temperatures up to 65 °C for two weeks, where uninfected grass plants and grass plant infected by the virus-free isolates died. A virus-free fungal isolate was then re-infected by CThTV, underwent the same heat treatment, and found to confer the same level of heat tolerance as the wild-types. They were also able to infect tomato plants with the fungus-virus combination and heat tolerance was also conferred to these (Márquez et al., 2007).

Endophytic fungi can be deleterious, neutral, or beneficial to their plant hosts and viruses have been found that can attenuate the pathogenicity of fungi to plants. One of the most famous examples is the case of chestnut blight, *C. parisiitica* (Nuss, 2005) and control of this pathogen is currently maintained in Europe by an attenuating virus (Anagnostakis, 1982). The majority of mycoviruses (viruses that infect fungi) have dsRNA genomes, are persistently transmitted, and typically do not produce symptoms in their fungal hosts (Buck, 1988; Ghabrial et al., 2015). The authors suggest that the lack of symptoms evident in fungi infected by viruses could indicate that other mycoviruses may be at least partially responsible for the beneficial effects of fungal infection observed in some cases of fungal-plant symbiosis (Márquez et al., 2007).

\[b) \quad \text{Virus Infection Improves Host Tolerance to Drought and Cold}\]
Several acute, RNA viruses and numerous economically important crop plants were used in this study evaluating the effect of virus infection on drought and cold tolerance of hosts (Xu et al., 2008). Drought tolerance of beet (*Beta vulgaris* L. cv. Detroit Dark Red), pepper ‘Marango’ (*Capsicum annuum* L. cv. Marango), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai cv. Crimson Sweet), cucumber (*Cucumis sativus* L. cv. National Pickling), tomato (*Solanum lycopersicum* L. cv. Rutgers), *Solanum habrochaites*, zucchini (*Cucurbita pepo* L. cv. Elite) Chenopodium *amaranthicolor*, *Nicotiana benthamiana*, and tobacco (*Nicotiana tabacum* L. cv. Xanthi nc) was tested after infection by *Cucumber mosaic virus* (CMV). Drought tolerance was also evaluated in *Nicotiana benthamiana* infected by BMV (*Brome mosaic virus*), TMV (*Tobacco mosaic virus*) and TRV-GFP (*Tobacco rattle virus* clone carrying a fragment of the GFP gene), in order to compare the effects of different viruses in the same host plant on drought tolerance. Drought tolerance of rice (*Oryza sativa* cv. IR-8) was evaluated in plants infected with BMV. The authors indicate that these viruses were chosen, in part, because they represent a diversity of host ranges; CMV is a virus with a broad host range, TMV and TRV have intermediate host ranges, and BMV has a limited host range (Xu et al., 2008). Beet plants infected with CMV were tested for cold tolerance as well as drought tolerance.

Seedlings of *Nicotiana benthamiana*, *Chenopodium amaranticolor*, and tobacco were inoculated one month after germination or mock inoculated. All others were inoculated at the two-three leaf stage. The plants were subsequently grown individually in pots in growth chambers. Watermelon, cucumber, tomato, pepper, *C. amaranticolor* and *S. habrochaites* received full water for 2 days, eight days after inoculation. *N.*
*benthamiana* and tobacco received two days of full water 14 days after inoculation. These plants were then moved to dry flats. Rice plants received full water treatment until 28 days after inoculation and were then moved to dry flats. The number of days passed since water was withdrawn was recorded both for when plants had wilted tips and when they “collapsed” (Xu et al., 2008). The plants were then watered regularly for 1-2 weeks. Water content (g water/plant) at harvest and water loss rate was calculated for the beet plants. For the cold tolerance experiment, beet plants at the 2-3 leaf stage were inoculated with CMV or mock inoculated. The plants were moved to a growth chamber 28 days after inoculation set to 16 hours daytime at 15°C and -2°C for 8 h dark. This was followed by another 15°C, 16-hour day, followed by -4°C, 8-hour night. All experiments were repeated at least once, with most repeated several times.

All plants except for the tobacco plants had delayed onset of drought symptoms by 2-5 days (Xu et al., 2008). Beet plants were subjected to further study. Four days after withholding water, mock-inoculated plants had collapsed while new leaves of CMV-infected plants remained turgid. Water was withheld for an additional 4 days and then plants were watered regularly for one week. All of the CMV-infected plants recovered and grew new shoots. Only 30% of mock-inoculated plants did. Measured water content of infected plants was higher and infected plant leaves lost water more slowly. In the experiment involving *Nicotiana benthamiana* infected by several different viruses (each plant infected with one virus), virus infection delayed drought symptoms by 2-5 days compared to mock-inoculated controls. Rice seedlings infected with BMV had delayed onset of drought symptoms an average of 9.7 days. By this time, mock-inoculated plants were entirely wilted. A regular watering regime was restored for two
weeks. All BMV-infected rice plants and no mock-inoculated plants recovered. BMV does not produce severe disease symptoms in rice. In the experiment involving CMV-infected plants and cold stress, CMV-infected plants were cold-tolerant while all of the mock inoculated plants died (Xu et al., 2008).

Metabolic analysis of rice plants subjected to drought stress revealed a change in accumulation of only 43 metabolites in BMV-infected rice, while mock-inoculated plants showed a difference in 87 metabolites (Xu et al., 2008). CMV-infected beet plants subjected to drought stress similarly had fewer changes in metabolite levels compared to mock-inoculated plants. The authors claim this could indicate less sensitivity of virus-infected plants to drought stress compared to uninfected. Specific metabolites known for improving stress tolerance to plants (Xu et al. 2008 and references therein) were measured. Accumulation of trehalose, putrescine, and salicylic acid (SA) increased in CMV and BMV-infected plants. Ascorbic acid was increased in BMV plants and decreased in mock-inoculated plants subjected to drought stress. CMV-infected plants had greatly increased levels of sugars including melezitose, maltose and galactose, as well as anthocyanins. Therefore, the increased levels of these and other osmoprotectants and antioxidants were associated with improved drought tolerance (Xu et al., 2008).

V. Advances In Plant Virus Detection

Development of an enzyme-linked immunosorbent assay (ELISA) method for detection of viruses (Adams and Clark, 1977) enabled virus diagnostics to move from labor-intensive, time consuming processes requiring highly-trained specialists to a much simpler, shorter format. ELISA is the most common tool in certification, breeding, and quarantine programs and the industry standard for virus detection in crops (Boonham et
al., 2014). Disadvantages of ELISA are the costly development of required antisera, the frequent inability of antisera to distinguish between related viral strains, and the practical inability to test for more than one group of pathogens at a time (Boonham et al., 2014). After ELISA, the next major leap in viral diagnostics was the nucleic acid based test, polymerase chain reaction (PCR).

Kary Mullis and colleagues are credited with inventing PCR, describing the process for the first time in a paper published in the Science journal in 1985 (Saiki et al., 1985). PCR is the targeted amplification of a specific DNA sequence allowing detection and subsequent manipulation of the sequence. Real-time PCR, which reduces contamination through closed-tube assays, is the most widely adopted PCR variant and TaqMan® probes the most popular for diagnosing viruses (Boonham et al., 2014). Advantages of real-time PCR over ELISA are sensitivity/specificity, faster and cheaper set up for new viruses, and more general applications (bacteria, fungi, etc.) (Boonham et al., 2014). Whether one uses PCR or ELISA depends on specific circumstances. A recent study concluded that while there were slight differences in viruses detected, TaqMan® RT-qPCR and ELISA were equally good techniques for screening for known viral diseases (Schellenbaum, 2017). The most important limitation of both techniques is the need for prior knowledge about the particular virus being assayed in order to make appropriate antisera, probes, or primers. While ELISA and PCR will continue to have widespread applications in screening for known viruses, next-generation sequencing (NGS) is expanding our understanding of virus ecology and pathology by enabling discovery of previously unknown viruses.
Next-generation sequencing and the accompanying bioinformatics was first used in plant virology in 2009 in multiple studies to discover and identify new DNA and RNA viruses and viroids (Adams et al., 2009; Hadidi et al., 2016 and references therein). There are three central steps in the process; library preparation of nucleic acids to be sequenced, amplification of the nucleic acids to detectable levels, and parallel sequencing of “millions or billions” of DNA fragments (Massart et al., 2014). Bioinformatics then takes over making it all possible. De novo sequence assembly, matching generated sequences to each other, is what allows discovery of unknown viruses and viroids (Massart et al., 2014). Boonham et al. (2014) provide a review of various approaches and sequencing platforms utilized during NGS. This includes the dsRNA isolation technique followed up with cDNA synthesis used by Roossinck et al during the metagenomic study which showed most viruses in natural ecosystems are latent (Roossinck et al., 2010; Boonham et al., 2014). Some applications of NGS include disease etiology, viral population studies among wild and agricultural plants, fast sequencing of entire genomes, and elucidating plant response to viral infection (Prabha et al., 2013).

Recent metagenomic and ecogenomic studies made possible by the advent of NGS technologies are revealing the nature of viruses and expanding our conception beyond their role as pathogens. Metagenomic analysis involves sequencing pooled, larger-scale environmental samples while ecogenomics focuses on sequencing individual plants for all viruses (Roossinck et al., 2010). While it appears most viruses in natural environments are asymptomatic (Roossinck et al., 2010, 2015), some are clearly beneficial to their plant hosts and even known, disease-causing viruses may provide benefits depending on environmental conditions. Advances in sequencing technology
will lead to a rapid expansion of our knowledge of the ecological roles of viruses in both natural and managed ecosystems. As the field of agroecology develops and incorporates this knowledge, it can be applied to create agroecosystems that require fewer external inputs resulting in greater productivity with less environmental damage.

VI. Conclusion

The green revolution intensified agriculture production through improved crop varieties, better farm machinery, and development of synthetic fertilizers and pesticides. As a result, food production in many regions of the world increased without a corresponding increase in new land placed under cultivation. While these gains are impressive, fifty-plus years of high-input crop production systems has resulted in local and global environmental consequences. Food production increases are not sustainable using conventional agriculture practices. Agroecology bases agroecosystem design and management on ecological principles in order to replace external inputs with services provided by natural processes. Applying these principles to crop production systems can result in greater, long-term economic security for farmers and fewer environmental consequences.

Viruses have long been ignored in ecological studies and the field of agroecology is only recently gaining momentum as the impacts of conventional agriculture practices become clear. Consequently, little is known of the ecological functions of viruses in agroecosystems and the services they may provide to crop production systems. Biodiversity maintains ecological processes and viruses are important members of ecosystem biodiversity. There are beneficial, neutral, and negative interactions of viruses with crop plants and other ecosystem components, such as the soil microbiome. Further
research will continue to reveal the diversity of viruses, their role in ecosystem processes, and how other system properties affect virus behavior and distribution. Agroecology must incorporate this emerging knowledge in the design and management of agroecosystems to create systems in which internal regulation replaces external inputs.
Agroecology: Principles for the Conversion and Redesign of Farming Systems

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Received date: February 22, 2016; Accepted date: March 30, 2016; Published date: April 05, 2016


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