Exploring the landscape of plant viruses using plant feeding insects

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It is an exciting time to be working in biology, specifically agricultural ecology. Over the last decade, new and powerful nucleotide sequencing technologies have been developed, and costs associated with these sequencing technologies has continued to drop. As a consequence, biologists are starting to use these next-generation sequencing technologies to answer questions about large scale ecological problems with adequate and appropriate experimental design and sample sizes.

Recently, metabarcoding has emerged as a nucleotide-based technique that allows researchers to broadly study the diversity of eukaryotic organisms in the environment. Similar to metagenomics, which characterizes genetic material directly from environmental samples, metabarcoding combines DNA taxonomy with high-throughput DNA sequencing, and has enabled large-scale studies on diet, resource partitioning, and food web ecology. In irrigated crop agroecosystems, we would like to use metabarcoding and metagenomics techniques to study insect feeding as it relates to the epidemiology of insect transmitted plant pathogens.

A critical factor for a successful disease control program relies on a detailed understanding of the components which directly affect disease development. Having knowledge of the organism or organisms being targeted and how the environment influences disease development is vital to successfully managing disease. For insect-vectored plant pathogens, multiple tactics are often required to manage disease including exclusion (e.g., planting clean seed), eradication (e.g., good weed/volunteer control), and protection (e.g., avoidance in space or time, pesticides). Historically, control efforts have been placed on insect control or pathogen source reduction. However, a field's landscape context, the crop and non-crop habitats surrounding a field, also influence the risk for disease development. In many cases, assessing the risk for disease occurrence and/or severity based on landscape context is difficult.

For some insect-vectored plant pathogens, the habitats surrounding the crop of interest can affect disease occurrence and severity by 1) providing primary sources of pathogen inoculum, and 2) influencing insect abundance in some way (e.g., providing overwintering, feeding, and/or reproductive hosts). In theory, the amount of risk a landscape imposes on a crop can be estimated by quantifying 1) the abundance of plant species present in the landscape (i.e., percent cover), 2) the probability of a specific plant species to be infected with a specific pathogen (i.e., percent infected), and 3) the insect utilization of specific plant species. This risk estimate can be calculated for each plant species in the landscape and is sometimes called the virus inoculum potential (VIP) index. The VIP index can be calculated in different ways, but for a single plant species the basic calculation is as follows:

VIP = (Abundance (% cover) * probability of infection (% infected) * insect utilization [a]) x 100

[a] Note: insect utilization is a generic term; in the literature it has been measured in different ways and there is no generally agreed upon "correct" measurement

Essentially, the VIP index allows for ranking of plant species based on their epidemiological importance. Plants that are abundant, highly infected with a pathogen, and utilized by insects for feeding are more likely to be epidemiologically important than plants that are not. Integrated over a whole landscape, the VIP could help to identify risky landscape. However, estimating the components of the VIP index at a large scale is difficult, costly, and labor intensive. Finally, the proximity of non-crop habitat to the crop of interest also will relate to risk. A crop is more likely to be impacted by a non-crop habitat that is in close proximity than a non-crop habitat that is far away.

Important and often overlooked is the fact that plant virus research has focused on viruses that cause economic losses of crops, little knowledge exists about potentially emergent plant viruses which occur in non-crop hosts. Alternative plant hosts provide reservoirs for virus diversity. In fact, they may be important for the evolution (i.e., mutation, recombination, or reassortment) of viruses that later invade crops by providing a place for virus evolution to occur. The lack of knowledge about the species composition and abundance of viruses present in non-crop habitats and the potential risk for virus spread to susceptible crops is an important gap in our understanding of factors that lead to pathogen emergence.

In a pathosystem where an insect-vector is essentially a specialist (i.e. an insect species that utilizes one or a small number of closely related plant species for food, oviposition and shelter) the disease epidemiology is somewhat simplified. It is by no means easy, but at least we have a good idea of where to look for epidemiologically important pathogen sources. However, in pathosystems where a pathogen is vectored by multiple insect species or by one (or several) highly polyphagous insect species, insects that use multiple plant species as feeding hosts, our ability to identify epidemiologically important primary sources of pathogens is limited. In these cases, identifying epidemiologically important sources is a "needle in the haystack" problem. It is costly and takes a large amount of effort to adequately sample plants to identify virus/pathogen sources.

Epidemiologically important non-crop hosts are those plant species that can be infected by the pathogen AND are commonly infected by the pathogen AND are commonly utilized by the insect vector. They are the plant species that overlap between the host range of the pathogen and the host range of the insect.

Insect-vectors themselves may provide a key to reducing the size of the haystack in which the needle is buried. They represent a unique opportunity to not only reveal the plant virus diversity that occurs in the agroecosystem, but also reveal the crop or non-crop plant species that may harbor that diversity. Collectively, a population of a single insect species may have effectively probed many different plant species within the plant community. In doing that, the insect population may have ingested (i.e. sampled) and be carrying nucleic acids from both the plant host on which they were feeding and the pathogen, if it was present in the feeding host. In the scientific literature, this is referred to as "vector-enabled" sampling – using insects to sample the environment. However, by appropriately sampling insect populations arriving to our crops and using metabarcoding and metagenomics to effectively analyze the insect samples we may be able to discover 1) the plant species that were used by the insect prior to their arrival, 2) the diversity of plant viruses present in those plant species, and 3) if plant virus assemblages cluster in groups based upon host plant or insect-vector association.