Transmission biology of *Xylella fastidiosa*

We report on aspects of studies aimed to better understand the transmission of the bacterial pathogen *Xylella fastidiosa*.

The specificity of pathogen-vector-host interactions is an important element of disease epidemiology. For generalist pathogens, different pathogen strains, vector species, or host species may all contribute to variability in disease incidence. One such pathogen is *Xylella fastidiosa*, a xylem-limited bacterium that infects dozens of crop, ornamental, and native plants in the United States. This pathogen also has a diverse vector complex and multiple biologically distinct strains. We studied the implications of diversity in this pathogen-vector-host system, by quantifying variability in transmission efficiency of different *X. fastidiosa* strains (isolates from almond and grape genetic groups) for different host plants (grape, almond, alfalfa) by two of the most important vectors in California, *Homalodisca vitripennis* (glassy-winged sharpshooter) and *Draeculacephala minerva* (green sharpshooter). Our results suggest that pathogen, vector, and host diversity impose contingencies on the transmission ecology of this complex disease system. Studies aimed at the development of management strategies for *X. fastidiosa* diseases should consider the complexity of these interactions as they relate to disease spread.

We also described the transmission ecology of *Xylella fastidiosa*. First, we performed a meta-analysis of transmission studies of *X. fastidiosa* by its two most important vector in the Western U.S., the invasive glassy-winged sharpshooter and the native blue-green sharpshooter. The importance of vector number, pathogen acquisition period, and inoculation access period for transmission differed relative to each other and between the two species. We fit these transmission datasets to two biologically derived transmission models. One of these models, the Poisson, provided substantially better fit and produced estimates of *H. vitripennis* transmission efficiency that were dramatically lower than for *G. atropunctata*. We also conducted a separate pair of experiments that decoupled vector number from inoculation access period. These experiments supported the results of the meta-analysis. Interestingly, high vector loads not only increased transmission rate, but also shortened *X. fastidiosa* incubation period in grapevines. This work provides quantitative estimates of transmission of an economically important pathogen that is analogous to risk models for arthropod-vectored human and wildlife diseases. In addition, this work suggests that heterogeneous vector loads may accelerate the disease cycle, increasing the potential for secondary spread in vineyards.

The interactions between the economically important plant pathogenic bacterium *Xylella fastidiosa* and its leafhopper vectors have been poorly characterized. We used different approaches to determine how *X. fastidiosa* cells interact with the cuticular surface of the foregut of vectors. We demonstrated that *X. fastidiosa* binds to different polysaccharides with variable affinities, and that these interactions are mediated by cell surface carbohydrate-binding proteins. In addition, competition assays showed that *N*-acetylglucosamine inhibited bacterial adhesion to vector foregut extracts and intact wings, demonstrating that attachment to leafhopper surfaces is affected in the presence of

specific polysaccharides. In vitro experiments with several *X. fastidiosa* knockout mutants indicated that hemagglutinin-like proteins are associated with cell adhesion to polysaccharides. These results were confirmed with biological experiments, when hemagglutinin-like proteins mutants were transmitted to plants by vectors at lower rates when compared to the wild type. Furthermore, although these mutants were defective in adhesion to the cuticle of vectors, their growth rate once attached to leafhoppers was similar to the wild type, suggesting that these proteins are important for *X. fastidiosa* initial adhesion to leafhoppers. We proposed that *X. fastidiosa* colonization of leafhopper vectors is a complex, stepwise process, similar to the formation of biofilms on surfaces.

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