The Role of Leafhopper Feeding in Vector-Microbe-Plant Interactions: Manipulating the System for Pathogen Management

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The first part of the presentation, by Walker:

Feeding behavior plays a large role in the transmission of microbes by vector insects from host plant to host plant. Understanding the intricacies of feeding behavior provides insights that may lead to development of better control tactics of insect-transmitted pathogens by use of plant genotypes or agrochemicals that interfere with components of feeding behavior that are critical to the transmission of the pathogen. In the first part of this presentation, our current state of knowledge of feeding behavior of leafhoppers is reviewed. The most important advances in our understanding of leafhopper feeding behavior have been made using the electrical penetration graph (EPG) technique, and these types of studies will be emphasized. While all leafhoppers are classified as piercing-sucking feeders, there is great variation in feeding behaviors among leafhopper species, and even within species. Some are primarily phloem-feeders, some primarily xylem-feeders, and others primarily mesophyll-feeders. Some, like *Empoasca* species, have a repertoire of various feeding behaviors that they can vary depending on circumstances. Also, in the first part of this presentation, the role of feeding behavior in acquisition and inoculation of plant pathogens will be very briefly reviewed. Most of this work has been done with Sternorrhyncha, but lessons learned there are very applicable to transmission of plant pathogens by Auchenorrhyncha.

The second part of this presentation, by Bextine:

The development of molecular techniques that allow for detection of as few as one cell have allowed us to understand the movement of microbes on a cell for cell basis. Combining these detection capabilities with traditional feeding behavior studies has led to more accurate diagnosis of pathogen movement. The glassy-winged sharpshooter (*Homalodisca coagulata*)/*Xylella fastidiosa* (*Xf*) model system has been studied to describe transmission events in a quantitative fashion. Movement of *Xf* from one plant to another depends on the transmission of the bacterium from an infected host to an uninfected host by the insect vector. For transmission to occur, two major events have to occur, acquisition and inoculation. In these studies we determined behaviors and timed events that are associated with successful movement of the bacterium. Positive correlations were detected between acquisition events and total ingestion time or acquisition access period (AAP) length, but not increased number of probes. On the other end of the disease cycle, positive correlations were detected between inoculation of *Xf* and number of probes or inoculation access period (IAP) length, but not increased total ingestion time. Understanding these associations will allow epidemiology studies of inoculative insects to be more accurate and help develop a means of reducing the efficiency with which the pathogen is spread from an infected plant to a non-infected one.

Planthopper (Hemiptera, Fulgoromorpha) Feeding Behavior: History and Current Prospects with EPG

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Feeding behavior is fundamental in the process of phytophagous insect diversification (Caillaud & Via , 2000). The feeding behavior of phytophagous insects is responsible for both direct damage and the transmission of pathogens. Planthoppers are a biogeographically diverse, monophyletic clade with about 12,000 described species grouped into 18 families (O'Brien, L.B. 2002; Stroinski A& Szewedo J., 2002). About 30 species of planthoppers are reported as vectosr of plant pathogens, in such familes as Delphacidae, which transmit viruses (Nault & Ammar, 1989), or Cixiidae, which transmit phytoplasmas (Purcell, 1985). Transmission of circulative and non-circulative pathogens depends on stylet localization and feeding activities. Electrical Penetration Graph (EPG) is a very powerful method to study stylet penetration behavior of hemipterous insects (Van Helden & Tjallingii , 2000). Within the planthopper clade, 5 species of Delphacidae, all in the tribe Delphacini, have been EPG-recorded.

History of planthopper EPG recording:

Chang (1978) was the first to record a planthopper by EPG: i.e. the sugarcane planthopper *Perkinsiella saccharicida* Kirkaldy, with the very first AC system. Three major EPG phases were identified and correlated respectively with mesophyll, xylem and phloem tissue localizations via salivary sheath histology. Kimmins (1989) identified six different EPG phases from *Nilaparvata lugens* Stal on rice using the DC system (Tjallingii, 1985). Two EPG phases were correlated with phloem and xylem ingestion via analysis of honeydew production. Kimmins (1989) argued that planthopper EPG waveforms were different from those of aphids because the salivary sheaths of planthoppers were intracellular. Spiller (1989) observed by TEM that the maxillary stylets of *N. lugens* enter the sieve element accompanied by sheath saliva, unlike what has been observed with aphids, where the salivary sheath stops at the sieve element cell wall and only the stylets protrude into the cell.

We have studied the feeding behavior of maize planthopper, *Peregrinus maidis* (Asmead), taking advantage of the new possibilities to acquire EPG data via a microcomputer (Buduca & *al*, 1996). Statistical analysis of digital data showed that some physical parameters could be distinguished, especially three major EPG phases. Spectral analysis allowed us to show major frequencies related to muscular activities. The knowledge of planthopper feeding behavior revealed by EPG was used to study plant resistance. Indeed, EPG analysis showed that the Delphacini are essentially phloem feeders, and that the major effect of plant resistance is a reduced duration of phloem phase (Chang & Ota, 1978; Kimmins, 1989).

Current prospect of planthopper EPG recording:

The possibilities for spectral and time-frequency analysis of digital data were used to more rapidly differentiate ingestion from salivation activities in sieve tubes for *P. maidis*, vector of *Maize mosaic virus* and *Maize stripe virus*, both circulative and propagative viruses. Indeed, ingestion and watery salivation are essential aspects of the feeding behavior of piercing-sucking insects, respectively correlated with acquisition and inoculation of circulative viruses (Prado and Tjallingii, 1994). Based on our time-frequency analysis and the observation by transmission electron microscopy of the fine structure of stylet pathways, passive ingestion and salivation were differentiated. Morever, temporal and spectral analysis of digital data from EPG recording produced many physical signal parameters. We chose the most significant ones to separate EPG phases, and with them developed software (termed 'EPG-Soft') for automated recognition of EPG recording (Reynaud & *al.*, 2003). An example will be presented of the use of our software for the analysis of data for development of host plant resistance. Indeed, in some maize inbreds MStV and MMV disease incidences varied in relation to the cumulative number of inoculative planthoppers, and revealed a non-specific virus resistance in maize (Dintinger & *al.*, 2005). EPG analysis by EPG-Soft showed that cumulative time in phloem mostly explained the resistance-susceptibility status of these maize inbreds. These results are further discussed in a broad perspective in relation to study of planthoppers and other hemipterans with EPG.

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Cell-Rupture Feeding by *Empoasca* spp.: How It Causes Hopperburn and Plants Defend Against It

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Hopperburn is a noninfectious plant disease attributed to the direct feeding damage caused by *Empoasca* spp. leafhoppers (Cicadellidae: Typhlocybinae) (Backus et al. 2005). Symptoms include leaf chlorosis, stunting of stems, and wilting of terminals and leaves. Mechanical damage by the lacerating stylets is likely the initial trigger of the hopperburn cascade (Ecale and Backus 1999). Cell rupturing is the characteristic feeding strategy employed by all *Empoasca* spp. (Backus et al. 2005). Electrical penetration graph monitoring determined three different stylet penetration tactics comprise the cell rupturing strategy, namely, lacerate-and-sip, lacerate-and-flush, and lance-and-ingest. While all *Empoasca* spp. possess the same repertoire, variations of the tactics and the tissues in which they are performed influence whether or not the hopperburn cascade will be initiated. *Empoasca* salivary components amplify the severity of the wound response first initiated by mechanical damage. Thus, initiation of the cascade of plant physiological events that cause hopperburn is termed a saliva-enhanced wound response (Ecale and Backus 1995, 1999). Insects employing a lacerate-and-flush style of feeding secrete large amounts of watery saliva that readily diffuses from the site of secretion, thereby exposing cells within a localized region to the salivary components. Watery saliva from phytophagous Hemiptera contain hydrolyzing and cell wall degrading enzymes, such as amylase, cellulases, hydrolases, and proteases. The combination of mechanical damage and salivary stimuli results in localized necrosis within probed regions, enlargement and proliferation of phloem parenchyma and vascular cambial cells. Vascular bundles become disorganized, collapsed, and constricted, resulting in an accumulation of photoassimilates in the leaves (Ecale and Backus 1995, 1999). Overall, the vascular constriction that ultimately occurs as part of the saliva-enhanced wound response has considerable consequences on whole-plant physiology, such as reduced phloem and xylem translocation, photosynthesis, and nonstructural carbohydrates.

Plants can successfully defend themselves against hopperburn by altering the repertoire of *Empoasca* feeding tactics (Serrano and Backus 1998, Serrano et al. 2000). Elevated healing/compensatory responses to mechanical and salivary factors can also minimize hopperburn symptoms. An additional well-studied example of a plant defending itself against hopperburn involves alfalfa, *Medicago sativa* L., and the hopperburning potato leafhopper, *Empoasca fabae* (Harris) (Ranger et al. 2001a,b, 2002, 2004a,b, 2005a,b, Shockley et al. 2001). Glandular trichomes found mainly on the stem and petiole surface secrete nonvolatile fatty acid amides that act as behavioral deterrents to settling by the potato leafhopper (Ranger et al. 2004a,b, 2005a). The glandular trichomes provide an effective barrier to colonization by the potato leafhopper and provide a crucial source of resistance against hopperburn.

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