

Symposium – Auchenorrhynchan Feeding Processes

Background on Electrical Penetration Graph (EPG) Monitoring in the Study of Auchenorrhynchan Feeding Processes

Elaine A. Backus, USDA Agricultural Research Service, Parlier, CA 93648 USA, ebackus@fresno.ars.usda.gov

Studies of Auchenorrhynchan feeding processes have been carried out for many years, using a variety of methods. The earliest studies, in the late 1900's, relied heavily on light microscopy of hopper-probed plant tissues to indirectly infer stylet pathways and sheath termini by examining the intact salivary sheaths. Even the oldest of these studies often have visually beautiful pictures of plant tissues and sheaths. However, salivary sheaths alone provide only a snapshot view of feeding, taken after the events have been completed. Until the invention of electrical penetration graph (EPG) technology in 1964 (McLean and Kinsey 1964), there was no rigorously quantifiable means of studying the intricate details of stylet penetration in real time. Various rapid stylet activities, cell types penetrated, fluids ingested, valve and diaphragm movements, can all be visualized in real time by using this technique.

This presentation is the Introduction to the Symposium on Feeding Processes and Their Role in Hopper-Plant-Microbe Interactions. The purpose of this talk is to provide basic information on the principles and applications of EPG. This will provide sufficient background for the audience to understand and appreciate the results to be presented in several of the papers in the Symposium.

The basic principle of EPG is simple. An output wire from the monitor into the soil of a potted plant electrifies the plant with a low-voltage AC or DC signal. A very thin, gold wire is glued to the dorsum of a test insect using silver conductive paint. The tethered insect is then connected to the input of the monitor, and placed on the electrified test plant. When the insect inserts its stylets into the plant (to begin stylet penetration, also called probing), the circuit is closed and current flows through the stylets, the insect and into the monitor. The voltage of that signal is then measured across the input resistor of the monitor, which provides an accurate model of the voltage of the plant-insect interface. Feeding behaviors of the insect cause variable resistance to the applied signal. This changes the constant applied signal into a variable voltage that, when collected over time, produces a waveform. In addition to resistance, some waveforms (depending upon how high the input resistor value is) can also be generated by biopotentials within the insect or plant. These include plant cell membrane breakages and streaming potentials caused by charge separation of fluid flowing rapidly through narrow tubes like stylets. Each insect species has a unique set of waveforms that represent its stylet penetration behavior. After a series of correlation experiments have been performed to define the waveforms, EPG can be used to visualize stylet penetration in real time, at the instant it is occurring.

After initial invention and introduction by McLean and Kinsey (1964) of the original, AC version of the monitor, many years were spent improving the technology. Significant improvements were made by: 1) Tjallingii (1978) who developed the detection of biopotentials and invented a DC version still in very popular use today, 2) Backus and Bennett (1992) who devised a more modern and noise-free AC device, and very recently, 3) Backus, Bennett and Tjallingii (ms. in prep.) who have developed the first universal AC-DC monitor with switchable input resistors, to better match the inherent resistance of each insect species recorded, and thus provide a more optimum blend of resistance signals and biopotentials in the waveforms.

EPG has been extensively used for aphids in the 40 years since its invention, and has now allowed identification of the finest details of, for example, the mechanisms of pathogen transmission by these insects. Application of EPG has been slower for Auchenorrhyncha, with which it was first used in 1970 (Crane 1970) and published in 1978 (Kawabe and McLean 1978). Unlike aphids, whose behavior is quite stereotypical and almost unvarying from species to species, Auchenorrhynchan feeding and waveforms vary significantly among taxa. This has necessitated that the time-consuming correlation process be performed for each new species. It is hoped that this process can now be hastened with the advent of the new AC-DC monitor, and that fascinating details of hopper stylet penetration will soon be revealed.

The depth and rigor of analysis of feeding behavior that EPG allows has many applications to the topics of the Symposium. Several of the papers of the afternoon will explore these applications, and others, in depth.

References

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Functional Anatomy of the Alimentary Canal and Salivary Glands in Leafhoppers and Planthoppers and Their Role in Pathogen Transmission

Astri Wayadande, Department of Entomology and Plant Pathology, 127 Noble Research Center, Oklahoma State University, Stillwater, OK 74078, astri@okstate.edu;

El-Desouky Ammar, Department of Entomology, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH, 44691, USA, ammar.1@osu.edu

For successful insect transmission of circulative and propagative plant viruses or mollicutes to occur, these microbes must overcome several barriers in their vectors, most notably midgut and salivary gland infection and/or escape barriers (Ammar, 1994). Stylets, foregut (precibarium, cibarium), alimentary canal and salivary gland functional anatomy is described for leafhopper and planthopper vectors (Ammar, 1985; Backus, 1985; Wayadande et al., 1997). Examples are given for the use of transmission and scanning electron microscopy, confocal laser scanning microscopy, immunolabeling and other techniques, in studying the routes, transmission barriers, and accumulation or multiplication of some circulative/ propagative plant viruses (e.g. maize streak Geminivirus and maize mosaic Rhabdovirus) and mollicutes (e.g. *Spiroplasma kunkelii* and *S. citri*) in their leafhopper or planthopper vectors (Ammar and Nault, 2002; Ammar and Hogenhout, 2005; Kwon et al., 1999). Recent studies also demonstrated the retention sites of semipersistent and non-persistent viruses in their leafhopper or aphid vectors, respectively, and the role of the helper component proteins in binding these viruses to the cuticular lining of the foregut and/or the food canal in the maxillary stylets. Maize chlorotic dwarf virus, transmitted by leafhoppers, and several potyviruses transmitted by aphids are examples of these two groups of viruses.

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