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Review

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AC–DC electropenetrography: fundamentals, controversies, and perspectives for arthropod pest management

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Abstract

Studying the intimate association of arthropods with their physical substrate is both important and challenging. It is important because substrate is a key determinant for organism fitness; challenging because the intricacies of this association are dynamic, and difficult to record and resolve. The advent of electropenetrography (EPG) and subsequent developments allowed researchers to overcome this challenge. Nonetheless, EPG research has been historically restricted to piercing–sucking hemipteran plant pests. Recently, its potential use has been greatly broadened for additional pests with instrument advances. Thus, blood-feeding arthropods and chewing feeders, as well as non-feeding behaviors like oviposition by both pests and parasitoids, are novel new targets for EPG research, with critical consequences for integrated pest management. EPG can explain mechanisms of crop damage, plant or animal pathogen transmission, and the effects of insecticides, antifeedants, repellents, or transgenic plants and animals, on specific behaviors of damage or transmission. This review broadly covers the principles and development of EPG technology, emphasizing controversies and challenges remaining with suggested research to overcome them. In addition, it summarizes 60+ years of basic and applied EPG research, and previews future directions for pest management. The goal is to stimulate new applications for this unique enabling technology.

Keywords: arthropod-substrate interactions; electronic monitoring; electrical penetration graph; feeding behavior; egg-laying behavior; pathogen transmission; pesticides; host plant resistance

1 ARTHROPOD–SUBSTRATE INTERACTIONS

An organism's body size is arguably its most apparent and striking feature. However, our understanding of the world is frequently biased towards the size of human beings, neglecting the Lilliputian realm of arthropods despite their ecological and numerical importance.[1] Because size permeates all aspects of an organism's biology,[2–4] the naivete of simply scaling down the prevailing biophysical processes and phenomena from the scale of humans to that of arthropods can result in consequential mistakes, even if making for rather entertaining reading, as in Swift's *Gulliver's Travels* (1993, Parragon, Bristol, UK, reprinted from 1st Ed., 1726).

The stated bias compromises scientific appreciation of the intricate interactions between arthropods and their physical substrate (that is, any solid surface upon which they move, feed, mate, oviposit, or perform other behaviors). This is because when small organisms live in close proximity to their living substrate, as do arthropods, gravity loses its importance compared with the forces of cohesion, adhesion, and friction. The reduced influence of gravity compromises substrate adhesion, thus mandating life inside of hosts, or possession of structural modifications and/or secretions.[5] Such challenges faced by small organisms living in close association with any given substrate reinforce the need to understand the dynamics of such interactions. Observation of these dynamics is no small feat for researchers studying arthropods; thus, understanding the underlying mechanisms requires high

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resolution and fidelity in rigorous approaches. A specialized tool of the trade is necessary for such a purpose.

2 TOOL OF THE TRADE – ELECTROPENETROGRAPHY (EPG)

2.1 Birth and principle

Observation of visually discernible feeding behavior in chewing arthropods is simple compared with that of piercing–sucking arthropods. In the latter case, feeding behaviors take place within an opaque (food) substrate, plant or animal tissue, and thus are not directly observable. Furthermore, the process of plant pathogen transmission (that is, acquisition, retention, and inoculation) by many piercing–sucking plant-feeding species further reinforces the importance of studying their feeding behavior. Interest in transmission of plant viruses by the pea aphid, *Acyrthosiphon pisum* (Harris), motivated development of the first electronic system for monitoring aphid feeding (today termed electropenetrography or electrical penetration graph, both abbreviated EPG) between the late 1950s and early 1960s.[6,7] The first recordings were published in 1964,[7] and subsequently were correlated with simple stylet probing behaviors within the plant.[6]

The electrical principle of EPG is simple. After a gold wire is glued onto the arthropod with conductive adhesive, the arthropod is connected to the input of a head stage amplifier, which is in turn attached to a control box that also electrifies the substrate surface, or host, where the arthropod is placed (Fig. 1a). When the arthropod touches or inserts part of its body onto/into the substrate, both become part of an electrical circuit. By Ohm's Law, measurement of changes in electrical voltage can be measured as a proxy for electrical current flowing between interacting arthropod and substrate.[6,8]

The primary carriers of electrical signal between arthropod and substrate are ionized fluids. When the arthropod probes or bites (or its claws/feet scratch) the surface or host tissues, fluids flowing through the mouth parts (or across the feet/claws scraping a plant) close the circuit, allowing the recording of voltage fluctuations corresponding to the fluid dynamics taking place. This output voltage is further amplified through a secondary circuit and sent to a display (or output) system. Changes in voltage over time form discernible waveforms that can be interpreted as behaviors occurring otherwise invisibly in or on the substrate. For a more detailed explanation of EPG electronics, see a recent review,[11] and references cited therein.

2.2 EPG development

Advancement of EPG instruments followed the available electronic technology through time. First-generation EPG started with glass-tube amplifiers in the late 1950s, progressing to early solid-state transistors by the 1960s using alternate current (AC) and low amplifier sensitivity [or input resistor (Ri) of 10^6 Ω] in the primary circuit.[7] This early AC monitor [called then the 'electronic measurement (or monitoring) system (EMS)'] was radio-technology inspired and led to a series of subsequent designs during the 25 + years of its use, via changes in the secondary circuit (either in amplifiers, or filters, or both).[6] The limitation of these nonetheless pioneering designs was their inability to record tiny, fluctuating biological voltages (or biopotentials), termed electromotive force (emf) in EPG science,[8,9,11] which are generated by the arthropod interacting with its (usually plant) substrate. AC monitors were revolutionary for their time, but treated the gestalt of the arthropod and its substrate in the primary circuit as though they were strictly a variable resistor (Ra).

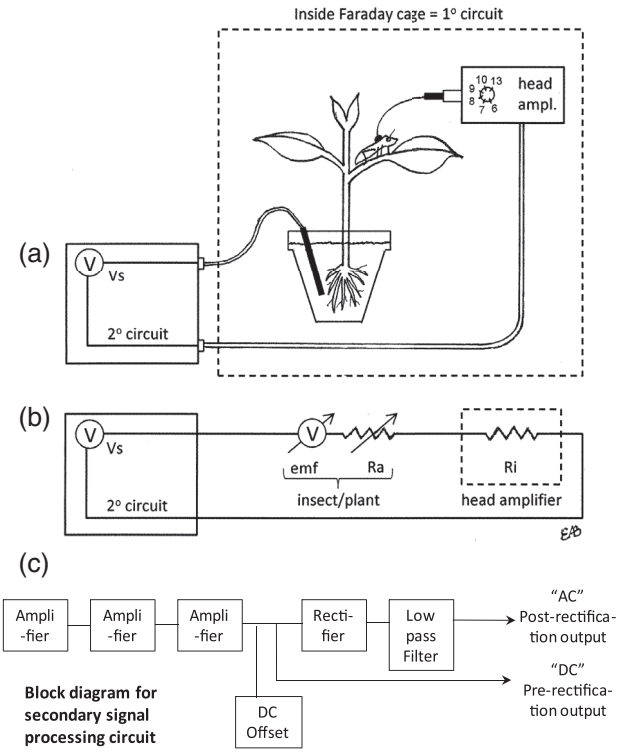

Figure 1. Diagrammatic representation of the primary and secondary circuits of an EPG monitor. The head stage amplifier is that of an AC–DC electropenetrograph because it has switchable input resistors. (a) Realistic model of the insect on electrified plant. (b) Electronic block diagram of the primary (1°) circuit, including variable biopotentials (emf) and variable resistance (Ra). 2°, secondary circuit (i.e. signal processing circuitry); head ampl., head stage amplifier; emf, electromotive force (biopotential); Ra, insect (e.g. aphid) resistance; Ri, input resistance/impedance of the head amplifier; *Vs*, source voltage. Figure derived from an original drawing by G. Walker.[8] Figure used with permission of American Phytopathological Society, from Backus[9]. (c) Block diagram for the secondary signal processing circuit. Open-access figure from Backus and Shih[10].

Advances in electronic technology led to the development of more sophisticated and affordable amplifiers and recording devices, setting the stage for the second-generation EPG, developed during the late 1970s. The technology was improved for aphids by changing to DC (direct current) applied signal, using operational amplifiers (op amps), a Faraday cage to control noise, and either FM tape recorders or rapid-response strip chart recorders as output devices. The latter was a big improvement compared with the slow-response strip chart recorders used with the AC monitor. The newer design also occasioned re-naming the technology as 'electrical penetration graph (EPG),' also known as the DC monitor (or system).[12] Nonetheless, the most important modification advanced in the DC system was its higher amplifier sensitivity or Ri, either 10^8 or 10^9 Ω for the standard amplifier, or 10^{11} to 10^{13} Ω for a special amplifier for emf (see below), allowing recording for the first time of tiny emf signals.[8,12]

In addition to development of the DC monitor, the modern theoretical foundations of EPG science were established at this time by identifying and explaining the R and emf components of signals, in series within the EPG primary circuit (Fig. 1b).[8,11] R components are caused by: (i) physical resistance to electrified fluid flow,

or (ii) different levels of fluid conductivity, such as for saliva *versus* dilute plant sap. Electromotive force (emf) components are tiny biopotentials generated by: (i) stylet breakage of plant membrane potentials, or (ii) streaming potentials generated by electrified fluids squeezed through narrow capillary tubes such as the food canal in stylets. R components are dependent on the applied signal, whereas emf components are inherently generated in the arthropod–plant interface, and thus independent of applied signal.[8,11] The higher Ri levels of the DC monitor allowed the simultaneous detection of not only the R components already detected by the previous AC monitor, but also the emf components (Fig. 1b).[8,11] R and emf are blended together in the EPG output waveform and the identification of these electrical origin(s) for each waveform greatly aids in understanding its biological meaning.[11]

Continuous innovations in computers and electronics greatly influenced EPG development. Integration of computers with EPG in the 1990s led to the use of computerized analog-to-digital waveform display, magnifying waveform details by more than 30× compared with the original AC monitor. Also, a dichotomy developed in EPG research during the 1980s and 1990s, with specialization in the use of AC monitors for medium-to-large insects (especially auchenorrhynchans like leaf-, plant-, and treehoppers, as well as spittlebugs), and of DC monitors for smaller hemipteroid species [especially aphids, whiteflies, psyllids, and other sternorrhynchans, as well as thrips (thysanopterans)]. This specialization lasted until around 2000, when the manufacturing of AC monitors ceased. Thereafter, EPG research relied on DC monitors, which are used still for most EPG research. However, a third generation of EPG instrument, the AC–DC electropenetrograph, is rapidly growing in its acceptance and applications.[11,13]

2.3 AC–DC electropenetrograph

The design of the third-generation EPG monitor was inspired when the theory of the (now-named) R/emf responsiveness curves (Fig. 2) was explained.[11] These curves graph the relationship between R and emf for each species. Their calculation showed that a Ri of 10^9 Ω provided the best 50:50 balance point of R and emf components (also known as the fixed inherent resistance, or Ra) for average-sized aphids. By contrast, slightly lower Ri (10^8 Ω) was best for larger aphids and slightly higher Ri (10^{10} Ω) was best for smaller aphids[11,12,14] (solid curves, Fig. 2). In extension of this idea, it was hypothesized that even lower Ri levels would be needed for even larger insects, such as large sharpshooter leafhoppers (Cicadellidae: Cicadellinae) and heteropterans (dashed curves, Fig. 2). Thus, a range of Ri levels from 10^6 to 10^{10} Ω (plus 10^{13} Ω for pure emf) would be needed to expand the usefulness of EPG to arthropods outside small-sized hemipteroids.[11] Size may not be the only criterion for an insect's inherent resistance; soft- *versus* hard-body, surface-to-volume ratio, and other characteristics may also be involved (although see further discussion below). Nonetheless, it took 22 years of iterative designs and evaluation before the new monitor design was published.[11,13] The commercialized, four-channel version incorporated and optimized all the functions of both the first- (AC) and second-generation (DC) monitors (Fig. 1a,c). The AC–DC monitor design allowed: (i) selection of Ri levels in the above range; (ii) choice of either AC or DC applied signal; and (iii) then-more-up-to-date electronics in commercially printed, circuit boards.[11] Both the currently marketed AC–DC and DC monitors have further updated their electronics to newer technology, which allows most of the instrument to be machine-built and sturdier.

Thus, the third-generation, AC–DC EPG monitor (more properly, electropenetrograph) combines the electronic advantages of both previous generations of EPG monitors without their disadvantages. Flexible equipment settings can now allow researchers to tailor the instrument to best match the needs of any arthropod recorded, of any size. It also allows development of waveform libraries of output signals at different Ri levels and more precise recognition of R and emf components. Besides the flexibility of AC–DC EPG, the settings can be designed to output AC- or DC-monitor-type waveforms that are completely backwards-compatible with those of previous instrument designs. This means that

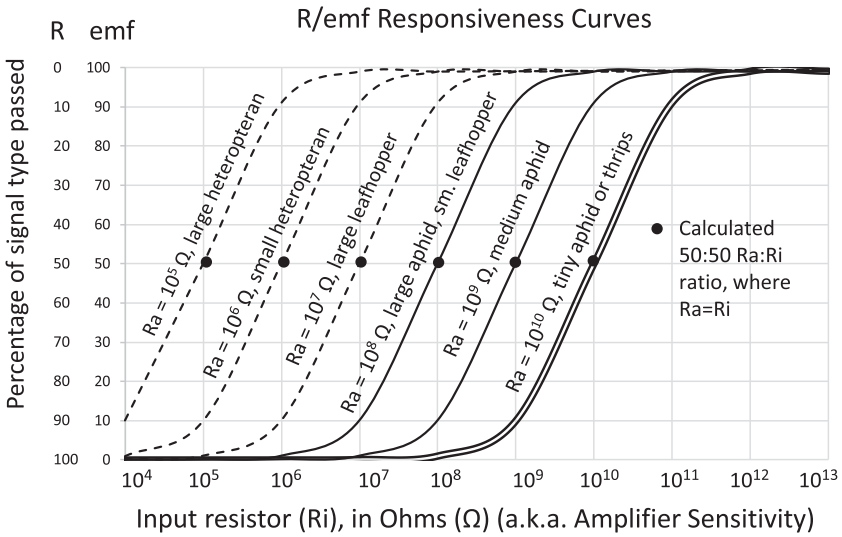

Figure 2. R/emf responsiveness curves. Percentage of signal types passed by the head stage amplifier is plotted over Ri level(s) chosen for recording. Most lines represent responsiveness to emf; however, Tjallingii's responsiveness to R is drawn parallel to the emf line for Ra = 10^{10} Ω, by inverting the R axis. Data are replicated from calculations by Tjallingii (solid lines) and supplemented by Backus's hypotheses (dashed lines and suggested interpretations of Ra levels for emf responsiveness, in text parallel to lines). Dark circles are theoretical calculations of the 50:50 Ra:Ri ratio, see reference[11] for details. Figure used with permission of Oxford University Press, from Backus, Cervantes, Guedes, Li, and Wayadande.[11], permission conveyed through Copyright Clearance Center, Inc.

AC–DC waveforms can be directly comparable with previously published AC or DC waveforms for the first time in EPG history.[11] Despite the versatility of the newer AC–DC design, the DC monitor is still used and preferred by many researchers. Thus, some controversies between the two systems still linger, and some challenges for future EPG research still remain.

3 CONTROVERSIES AND CHALLENGES

3.1 Controversies: Ri levels and the R/emf responsiveness curves

Both operational and scientific controversies exist between users of DC *versus* AC-DC monitors, but they have seldom been debated in print,[15,16] and not recently. Herein, we will explain and evaluate the most recent controversies from multiple perspectives and refer to or suggest research to answer remaining questions.

Experienced researchers using DC monitors wonder whether they should switch to using AC–DC monitors, or add them to their research. Despite the advantages of the AC–DC monitor described above, there are definite advantages of using the DC system. For example, the most popular DC monitor, the Giga8 (EPG Systems,[1] Wageningen, The Netherlands; https://epgsystems.eu/) has eight channels, therefore eight insects can be recorded (all monitors use one channel per insect) rather than the four channels available in the more electronically complicated AC–DC electropenetrograph [EPG Technologies,[2] Gainesville, FL, USA; available from Andrew Dowell (andygator3@gmail.com)].[11] Thus, there is currently less cost per channel for the DC monitor and more insects can be easily recorded in one experiment. In addition, for tiny insects like aphids and most other sternorrhynchans, the DC monitor's fixed Ri of 10^9 Ω is a good working level of sensitivity that produces clear, identifiable waveforms. There also appear to be no deleterious effects of the DC applied signal on tiny insects, and the purchase includes useful, semi-automated measurement software for aphid and related waveforms (see further discussion below on both points). For sternorrhynchan-specialized researchers who intend to always study tiny insects the size of most aphids or smaller, the DC system is a good, working instrument. It also may be the best choice for novices to become proficient with EPG by starting with a simpler-to-operate system than the AC–DC monitor.

For experienced researchers interested in broadening the range of questions that can be investigated, or for those studying arthropods larger than aphids or a broader diversity of differently sized arthropods, the AC–DC monitor provides more advanced, flexible settings. Nonetheless, some DC monitor-users are still unconvinced that different Ri levels are needed for large insects. Some doubt the science behind the R/emf responsiveness curves (Fig. 2), despite the fact that the emf curve was used to determine the fixed operational Ri level used in the DC monitor design.[11,14] No direct measurements similar to those with aphids[12,14] have been made yet with other, larger insects.

That said, no evidence has yet been published that disputes the R/emf responsiveness curve theories, neither the early work[14] nor other researchers' later work with insects larger than aphids. The best empirical evidence so far in support of the new hypothesis are the findings from eight waveform library studies published (to date) for non-aphid species using the AC–DC electropenetrograph.[17–20, 22–25] In every case, they reveal exactly what the responsiveness curves hypothesize, that is, that R-containing waveforms are best seen at low Ri levels, emf-containing waveforms are best seen at high Ri levels, and blends of R and emf are seen at levels in between. In other words, the organized changes in appearances of waveforms, as switches are made from Ri to Ri, are interpretable based on the responsiveness curves. In addition, the R:emf 50:50 balance of waveform appearances always occurs at lower Ri levels for large insects than the balance point of average aphids, 10^9 Ω. For example, several stink bug (Heteroptera: Pentatomidae) species have now been recorded with the AC–DC electropenetrograph, and waveform libraries published.[20–22,25] A standardized Ri setting of 10^7 Ω has been established for the best balance of R and emf.[21]

Additionally, a few previously published papers using DC monitors (with a fixed Ri of 10^9 Ω) to record relatively large sharpshooter leafhoppers or planthoppers conclude that all described waveforms are 100% emf.[26,27] This finding seems unlikely because no auchenorrhynchan feeding can plausibly lack an R component; all hemipterans salivate and/or move their mouth parts during feeding, which would give rise to R components. It seems more plausible that R components occur but are not detected by a fixed Ri of 10^9 Ω. In other words, for larger insects, Ri of 10^9 Ω cannot detect R-dominated waveforms because, according to the responsiveness curves, very little (if any) R component is detectable at that Ri level. A finding of 100% emf is exactly what is predicted by an R/emf responsiveness curve whose midpoint of 50:50 R:emf is $< 10^9$ Ω, or shifted to the left for large insects. Indeed, recent research has concluded that most sharpshooters have an R:emf balance point of 10^7 or 10^8 Ω, depending on body size.[18]

For purposes of this review, the senior author contracted a consulting PhD electrical engineer [Interdisciplinary Consulting Corporation (IC2), Gainesville, FL, USA] to review the R/emf responsiveness curve literature and evidence. The engineer performed an analysis of a complex, lumped-element numerical simulation model of the EPG system, including unintended ('parasitic') capacitances, inductances, and resistances from both the electronics and experimental system. Special attention was paid to whether such parasitics could combine to filter out signals of interest. It was concluded that the simple two-element R/emf model (Fig. 1) is valid, as long as the EPG user and instrument designer are careful to minimize capacitances that could significantly attenuate high-frequency components of the EPG signal. For example, wiring method should be perfected to avoid 'capacitance tails' on diagnostic waveforms like the aphid potential drop. Representative body lengths and mouthpart canal diameters of test hemipterans from the literature were input to another numerical model to calculate hypothesized Ra levels and current densities for these species (below). Resulting Ra levels ranged from 10^8 to 10^9 Ω for aphids ($n = 14$ species), 10^6 to 10^8 Ω for leafhoppers and a spittlebug ($n = 5$), and all heteropterans were 10^7 Ω ($n = 6$) (therefore matching the empirical findings described above, but in conflict with the hypothesis in Fig. 2). The magnitude of Ra was, indeed, inversely proportional to body size and stylet canal diameter. After these experimental Ra levels were analyzed using the model, results supported the hypothesis that R/emf sigmoidal curves would move to the left with increasing arthropod size.

The above simulation model plus early waveform libraries strongly support the science underlying the R/emf

[1]Giga8 DC monitors can be ordered from EPG Systems, at its website: https://www.epgsystems.eu/

[2]AC-DC 4-channel electropentrographs can be ordered by emailing the CEO of EPG Technologies, Inc., Andrew Dowell, at andygator3@gmail.com.

responsiveness curves and AC–DC instrument. Further clarifying research to measure voltages derived from actual EPG recordings, similar to the earlier study with aphids,[11,14] should also be performed, so that this controversy about R/emf responsiveness curves can be fully laid to rest.

3.2 Controversies: existence and importance of electrode potentials in EPG output signals

One characteristic of the early DC monitor was output voltage drift, wherein the baseline and all waveforms carried on it would gradually shift position over time, usually upward if DC+ signal was applied to the plant. This drift could become very large (hundreds of mV) after long recording durations. It was attributed to the gradual accumulation of electrode potentials,[14] that is, the spontaneous generation of galvanic (battery-like) voltages because a combination of metals (copper, zinc, silver, and gold) are used for plant and insect electrodes in EPG. This explanation has been firmly disproven. As explained in more detail elsewhere,[13,15] the type of operational amplifier (op amp) used in the early DC monitor were notorious for adding artifactual voltages that cause the same type and magnitude of drift to the output signal, leading to the use of coupling capacitors in the Missouri AC monitor[28] to prevent voltage drift artifacts. Later, higher-quality, instrumentation op amps, now used in both the modern DC and AC–DC monitors, have removed those artifactual drifts. In actual tests, the AC–DC monitor output drifted < 2 mV with maximum amplification in over 72 h of continuous recording.[11] The IC2 consulting engineer confirmed that appreciable galvanic/electrode potentials would only develop at extraordinarily high experimental temperatures that are not likely in entomological laboratory settings. Thus, this topic is no longer controversial because the issue has been resolved electronically.

3.3 Controversies: AC *versus* DC applied current effects on subject arthropods

The AC–DC electropenetrograph is designed to output identical waveforms at each Ri level, regardless of whether AC or DC applied signal is used (assuming the offset knob is properly used to remove rectifier fold-over when AC is applied).[10] Nonetheless, sometimes there are slight but interesting differences between AC and DC applied signals. Despite the monitor design similarities, subject arthropods are exposed to different electrical signals in each case: AC or DC. When the AC–DC monitor was first designed, one goal was to make available both AC and DC applied signals to achieve backwards compatibility with all previous AC- and DC-monitor waveforms. However, then-surprising observations were made when testing AC *versus* DC applied signals to study larger-sized hemipterans. Feeding behaviors of very large hemipterans like glassy-winged sharpshooter, *Homalodisca vitripennis* (a very large leafhopper, body length 12 mm) and heteropterans such as stink bugs (11–16 mm body lengths) could be highly disturbed by DC applied signals. Heteropterans became agitated when high DC applied signals (>300 mV) were used. Most sought to leave the plant; large heteropterans often broke their wires. *Homalodisca vitripennis* usually froze in place when exposed to any DC signal >10 mV, unwilling to probe despite long starvation times. Also, *H. vitripennis* (which, like most sharpshooters, prefer to stand downward-facing when probing plant stems), often reversed their stance and probed facing upwards when experiencing DC but not AC applied signal (Backus EA, personal observation). There is, at present, no explanation for this change in sharpshooter behavior.

The above observations were thoroughly tested in a quantitative study using two, 4 × 4 factorial AC–DC EPG experiments with *Lygus lineolaris*.[29] The effects of four Ri levels (10^6 to 10^9 Ω) *versus* four voltage levels (2, 60, 150, and 250 mV) were compared in the first experiment with AC applied signal, in the second with DC. Results showed that *L. lineolaris* fed using both monitors. However, there were highly significant differences in feeding variables among almost all Ri levels and voltages for DC, especially at Ri 10^9 Ω. By contrast, AC applied signals caused almost no differences among Ri levels or voltages, except at the extremes of Ri 10^6 Ω or 250 mV. Overall, insects on DC-applied plants spent an average of only 30% of their time in stylet probing, whereas AC insects spent 40% probing. The study concluded that low AC voltages were less disturbing, and that DC applied voltage was not recommended for EPG recordings of *L. lineolaris* and other large heteropterans.[29] Despite this recommendation, a recent study successfully used DC EPG for waveform characterization and quantitative studies of brown marmorated stink bug, *Halyomorpha halys*.[30,31] Of a large number of variables tested between treatments, only an unusually small number were significantly different.[31] However, there is no way to ascertain whether DC applied voltage affected the feeding of these stink bugs without using an AC–DC monitor to compare among voltage types and magnitudes.

It can be argued that the above *L. lineolaris* study did not directly, statistically compare AC *versus* DC in one experiment. To achieve that, a second study has been performed with *Bagrada hilaris* using two, 2 × 2 factorial experiments. Two Ri levels (10^7 or 10^9 Ω) *versus* AC or DC were compared in both experiments. The first experiment used 50 mV of applied signal, and the second used 550 mV. Despite low sample sizes, the as-yet unpublished results showed several significant differences between AC and DC when the applied signal was 550 mV, but almost no differences with 50 mV (Tuelher E, Backus E, Lucini T, Ebert T, Panizzi A and Oliveira E, unpublished data), suggesting that DC could be safely used at very low voltage and moderate Ri levels, but not at high voltage and/or Ri 10^9 Ω. Thus, it is likely that Ri and applied voltage type and level interact to cause varying effects on heteropterans and probably other large plant-feeders, although effects are magnified when 10^9 Ω and DC applied signal are used.

Almost no literature exists on the effects of electrical signals on insect tissues, either piercing–sucking or chewing feeders. Therefore, speculation must be based on research with human subjects. Extensive discussion on this topic is referenced here.[29] To summarize, AC signals cause irritation (although less as frequency increases), whereas DC signals cause muscle tetany (or freezing).

It is possible that any insect could be negatively affected by applied signals, especially DC, if a sufficient current density were achieved through its stylets. However, by Ohm's Law, current is dependent on voltage applied to a resistance. We hypothesize that small sternorrhynchans like aphids have a high, fixed inherent resistance (Ra) to the flow of electrified fluids from the plant because their stylet food and salivary canals are narrow and bodies small. Thus, regardless of applied voltage level, only a low current density will develop in their stylets and body. This is especially true with high input resistor levels such as 10^9 Ω in the DC monitor. By contrast, very large stylet canals and body sizes are found in larger insects like sharpshooter leafhoppers and stink bugs, leading to a lower, fixed inherent resistance. Consequently, higher current density will develop in the stylets and bodies of these insects, especially at lower Ri levels, linearly

proportional to applied voltage level.[11] With the assistance of the consulting engineer, calculations of Ra and current density were estimated for representative aphid, leafhopper and heteropteran species. Results supported the above statements (Backus EA, unpublished data).

In summary, we speculate that the type of applied voltage matters less in the case of tiny insects because they are so resistant to current density that they probably cannot 'feel' the electricity. For large insects, current density from each type of applied voltage can matter because the electricity may be 'felt' by the insect. Although further tests can and should be performed, current research supports that AC *versus* DC differences in insect probing of tiny *versus* large insects are real and should no longer be considered controversial, at least for plant-feeding insects. Nonetheless, additional tests would be valuable, especially as new species are recorded for the first time.

Interestingly, new work with AC–DC EPG of mosquitoes (see below) suggests there might be a different story for blood-feeding arthropods.[24] There was no difference in appearance of mosquito EPG waveforms regardless of AC or DC applied voltage to a human hand on which the wired insects were feeding, as expected with the design of the AC–DC instrument. Also, there were no statistical differences in event durations of probing behaviors.[24] Although more testing will certainly be required to support these ideas, we speculate that perhaps plants are more 'transparent' to applied electrical signals than are vertebrates, so that plant-feeding insects could 'feel' the current if their fixed inherent resistance is low. Vertebrates are highly electrical organisms, with their own large, internally generated voltages. Perhaps addition of EPG's tiny applied signal is lost in the general electricity of the host. Or, possibly it is not sensed by mosquitoes because they are adapted to vertebrate electricity.

Accordingly, it is important that each researcher working with an arthropod species new to EPG, especially large arthropods, should investigate applied voltage type and intensity, especially in relation to Ri level, and choose the signal type that best preserves normal arthropod behavior.

3.4 Challenges for future AC–DC EPG research

Funding agencies and industry stakeholders support research and set priorities to determine effectiveness of various integrated pest management tactics and treatments, described further below. Thus, their highest priority is to use EPG as a tool to compare behaviors like feeding, oviposition, and walking/standing. The biggest hurdle to beginning such quantitative EPG studies is the time required to characterize and define the biological meanings of waveforms for a new species, termed qualitative studies. It is essential that qualitative studies be completed before quantitative studies can begin.

Accordingly, there is a pressing need to speed up the process of defining waveforms. Although the flexibility of AC–DC EPG settings allows the technology to spread to new pests, this does not change the established protocols for defining waveforms, which usually involve time-consuming histological and artificial diet studies to correlate every waveform with observable behaviors and stylet locations in the plant.[8] To help speed this process, development of a waveform library clearly identifies R and emf components of waveforms, allowing testable hypotheses for biological meanings to be proposed and more selectively tested. For example, defining the AC–DC EPG waveforms of *L. lineolaris* took 'only' 3 years. This is very short compared with the ~ 35 years required to define waveforms of aphids, ~ 15 years for *Empoasca* spp. leafhoppers, and ~ 10 years for sharpshooters, all of which admittedly perform more complicated waveforms than *L. lineolaris*. Nonetheless, it took less time for *L. lineolaris* partly because a waveform library was first constructed,[19] which then suggested a targeted follow-up study with histology and chemistry to define the most damaging waveforms[32] before a quantitative study was performed.[33]

To facilitate more rapid expansion of AC–DC EPG into new taxa and species, we need to further simplify and speed up the process of defining waveforms, R *versus* emf, and AC *versus* DC sensitivities. Current waveform libraries are designed to provide exhaustive comparisons among waveform appearances for different Ri levels and types of applied signal, within individual insect species. However, basic research is needed to develop even more rapid, standard research protocols that should be performed for each new species, prior to application of EPG for quantitative studies.

Such standard methods should include: (i) simpler waveform library protocols; (ii) steps for recordings and measurements to calculate a unique R/emf responsiveness curve for each species; and (iii) rapid quantitative measurements of AC *versus* DC applied signal effects. It may be possible to design a single experiment that achieves all of the above objectives. For example, a single, factorial experiment could compare two chosen Ri levels *versus* AC and DC applied signals at a moderately high voltage level, enough to generate negative effects from AC or DC, if they occur. Switches among slightly different applied signal levels during recording could be used to calculate the R/emf responsiveness curve. After recordings are completed, waveform appearances could be mined for a simplified waveform library, waveform amplitudes could be measured for R–emf comparisons. The responsiveness curve, and event durations could then be measured to test effects of AC *versus* DC on behaviors. Performance of this single, comprehensive experiment might very rapidly answer most questions for a new species.

There are other needs to maximize usefulness of AC–DC EPG for integrated pest management. Once waveforms are defined and quantitative comparisons begun, manual waveform measurement is tedious and time-consuming. Therefore, funding and research is needed to develop automated pattern recognition for measurement/annotation of waveforms via trainable artificial intelligence algorithms, as is already under development with some success for aphids with DC monitors.[34] For AC–DC EPG, tick waveforms might be a good place to start this development, because their waveforms are highly repetitive and simple (see below), similar to electrocardiograms, for which automated measurement has existed for many years. Higher funding levels often available for medically related vectors of human pathogens could provide resources to jumpstart such research.

Finally, as is common in science, a challenge for introducing EPG methods into new fields of study and new arthropods is terminology.[35] Translating EPG for use with blood-feeding arthropods (see below) requires correlating behavior and feeding process terminology among fields. For example, in mosquito research, a bite or probe is generally defined as beginning when stylets are inserted into the skin and ending at that point when blood ingestion begins.[36] In plant sap-sucking insects, a probe is defined similarly, but includes ingestion and any activity while stylets are inserted, ending at that point when stylets are withdrawn from the tissue.[35] Different hemipteroid researchers use penetration instead of probing, or feeding instead of ingestion. Feeding is an especially confusing term, either broadly meaning all aspects of probing/penetration or narrowly meaning ingestion only.[35]

Similarly, blood-feeders that are vectors of animal or human pathogens are described as acquiring and transmitting diseases, unlike vectors of plant pathogens, which are described as acquiring and inoculating pathogens. While glossaries of textbooks such as Brown[37] are important milestones, terminology in biological sciences can be highly variable, so it is important to define terms in most publications.

4 PERSPECTIVES FOR BASIC BIOLOGY

The early focus of EPG technology on studies of aphid feeding activities and plant pathogen transmission has continued to expand for the 60+ years since conception of EPG.[6,7,11] The evolution of EPG came with increased care in methodology and terminology, clarifications, and standardizations,[35] although controversies remain and were previously addressed. Because all three generations of EPG instrument design produced useful research results, both timely and timeless, the following review of topics draws upon studies using all instrument designs. Increasing popularity of EPG is understandable and expected, because the technique is not only versatile and informative but also economically affordable; at present, the complete equipment costs for either a DC system or AC–DC electropenetrograph and peripheral equipment is less than US$10 000. EPG also provides three main advantages: (i) enhancement of study output by running simultaneous observations of a large set of insects and with high resolution; (ii) information on the biomechanics of the arthropod–host interactive process (via recording of biopotentials and correlations with organism activity); and (iii) recording of a range of minute behaviors and associated processes not visually discernible.[38] As a consequence, from feeding behavior of aphids,[39] EPG has become the standard tool for studies of stylet probing by leaf- and planthoppers[9,40] recently expanding to heteropterans,[19,33,41,42] and now non-hemipterans and other behaviors with potential for much more research on blood-feeders, lapping and chewing insects.[24,38,43,44]

4.1 Feeding behavior of plant sap-sucking hemipterans

Prior to EPG, hemipteran feeding was usually studied via histological sectioning of salivary sheaths and gross symptoms of plant injury.[45] EPG provided the means of connecting those snapshots in time with a dynamic view of hemipteran feeding in real time. As a result, most of what is known about the intimate details of stylet probing behaviors has been developed via EPG, resulting in 672 published papers, to date. [Source: Backus EPG EndNote library, a complete collection of all English-language EPG papers from 1964 to 2010, including all pdfs (411 papers; available free by contacting the senior author), plus a Web of Science search for papers, using 'electrical penetration graph' or 'electropenetrography' as search terms, published 2011 to 16 July 2020 (261 papers).] Several reviews of this large body of work have been published for aphids,[8,34] whiteflies,[46] leafhoppers such as sharpshooters,[9,11] and heteropterans,[21] which can only briefly be summarized herein.

All hemipterans have four stylets in their stylet bundle or fascicle: a pair of outer mandibular stylets and a pair of inner, maxillary stylets. The maxillary stylets house two, separate canals to transport plant fluid (food) and saliva. Thus, the EPG applied signal is simultaneously carried by both plant fluid and saliva.

EPG waveforms and accompanying histological research have revealed methods of feeding that are phylogenetically related among the suborders of Hemiptera. The term 'hemipteran feeding strategies' was coined that described broad categories of feeding biology.[47] Both strategies and tactics within them were later re-described.[48] Two main strategies occur: (i) salivary sheath feeding, and (ii) cell rupture feeding. In sheath feeding, the insects secrete two types of saliva: watery enzymatic saliva and gelling (or sheath) saliva. Both types are usually secreted simultaneously, sometimes intermittently as the stylets are probed/penetrated through plant tissues, with gelling saliva hardening to leave a solid sheath behind in the plant tissues that traces the pathway of the stylets.[34,49] Cell rupture feeders secrete no (or very little) gelling saliva but large amounts of enzymatic saliva.[48]

All sternorrhynchans (e.g. aphids, whiteflies, mealybugs, psyllids) exclusively use the sheath feeding strategy, but with two main tactics: intercellular or intracellular penetration.[47] Intercellular penetration is when the narrow, flexible stylets follow the middle lamella between adjoining cell walls by mechanically and enzymatically spreading the walls apart as the wider stylets push through (most sternorrhynchans). Best known for aphids, EPG waveforms from intercellular penetration feature prominent 'potential drops' that represent brief intracellular punctures into cells along the circuitous stylet pathway. Waveforms can also identify and distinguish among phloem salivation, phloem ingestion, and xylem ingestion.[34,46] By contrast, intracellular penetration is when the stylets push directly through cell walls into their cytoplasmic interiors, or sometimes push between a cell wall and its adjoining cell membrane, along a straighter pathway.[50] In that case, potential drops do not occur, or only rarely if the membrane is delicately broken. Most sternorrhynchans use the intercellular tactic; a thorough, recent review concludes that only psyllids probe intracellularly (Shugart H, Killiny N and Rogers M, unpublished data). Despite these slight differences in probing behaviors among taxa, sternorrhynchan waveforms are remarkably similar and can usually be identified with the same waveform names and definitions.[46]

Such similarity in EPG waveforms within a suborder is not always the case. Waveforms of auchenorrhynchans (plant-, leaf-, tree- and froghoppers) are highly diverse, regardless of whether DC or AC–DC monitors are used.[6,51,52] Consequently, no overarching review synthesizing all recorded species has yet been written. That said, some similarities do exist. All auchenorrhynchans penetrate their stylets intracellularly. Almost all auchenorrhynchans use the sheath feeding strategy, with no variations in tactics. EPG waveforms for sheath-feeders are best defined for planthopper family Delphacidae (especially brown planthopper, *Nilaparvata lugens*)[53] and leafhopper subfamilies Cicadellinae (sharpshooters), and Deltocephalinae.[54] Despite great differences in waveform appearances, one outstanding finding is the existence of 'X waves' (although the waveform is not always called that) in recordings of almost all auchenorrhynchan species.[11,51–53,55] This waveform identifies first contact with a cell type (for example, phloem sieve element or xylem tracheary element) from which the insect may choose to ingest, and the sensory process of acceptance.[9,56] The best-studied auchenorrhynchan waveforms and X waves are for sharpshooters (Fig. 3)[9,11] and brown planthopper.[53,57] The only studied auchenorrhynchans that are not sheath feeders are in the leafhopper (Cicadellidae) subfamily Typhlocybinae. All studied typhlocybine species are cell rupture feeders. These insects are highly diverse in their feeding, employing four different tactics of rapid stylet movements and various cell types probed. Like their feeding

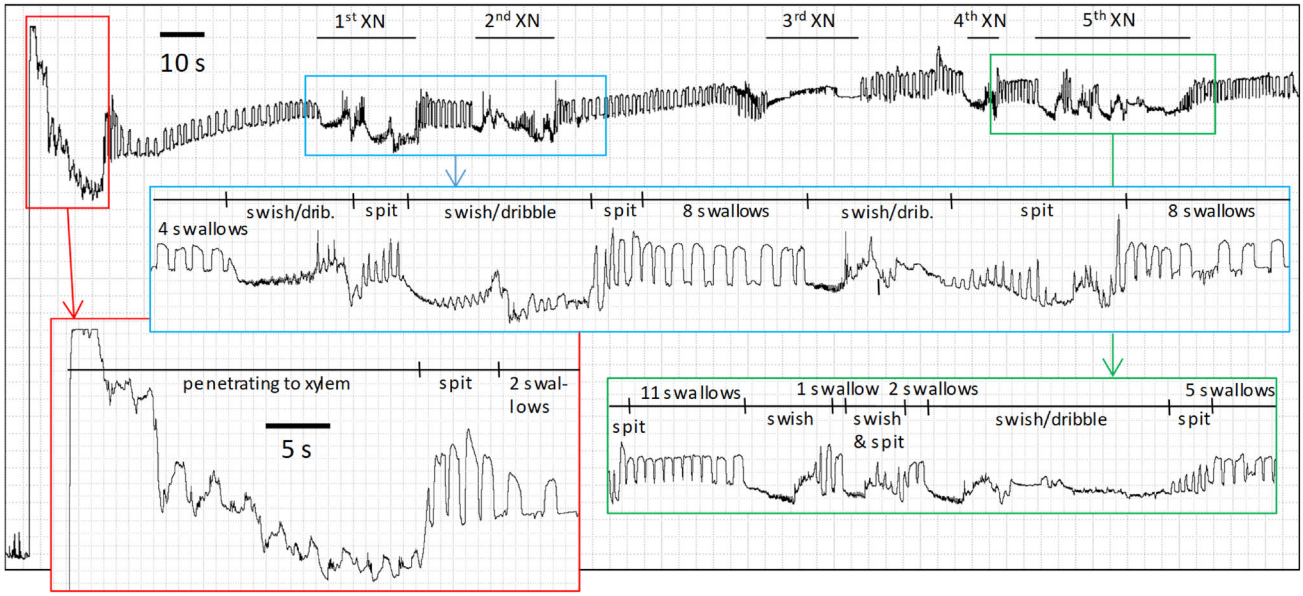

Figure 3. EPG waveforms from an early section of a single stylet penetration of a blue-green sharpshooter, *Graphocephala atropunctata*, on grape. Recordings were made with an AC-DC EPG at Ri 10^9 Ω, applied signal 25 mV AC. Figure components and meanings of the X wave (family XN labelled) are described. Sections of the compressed, main waveform trace (top) are enlarged in the red-, green- and blue-outlined inset boxes indicated by arrows. Labelled XN events represent swishing and spitting; tall peaks outside XN are XC events; each rounded peak represents a single swallow. Swishing by valves and pumps is combined with salivation; fluids are then egested (ejected) during spitting. Windaq gains: Main (black-outlined) box: compr. 30 (6 sec/div.), 16×; all colored boxes: compr. 5 (1 sec/div.), 16×.

strategy, their EPG waveforms are completely different from those of any other taxon.[48]

Like auchenorrhynchans, all heteropterans (true bugs) penetrate their stylets intracellularly. Unlike auchenorrhynchans, phytophagous heteropterans show strong waveform similarity by families. *Lygus* spp. are exclusively cell rupture feeders using a specialized heteropteran tactic called macerate-and-flush with varying degrees of slow-to-moderate stylet movement and copious enzymatic saliva secretion.[19,42] *Lygus* waveforms are unlike any seen for other heteropterans. By contrast, stink bugs (Pentatomidae) perform mixed sheath and cell rupture feeding (the only hemipterans known to do so) on a variety of plant structures. The newest, largest proliferation of AC–DC EPG research has identified waveforms and feeding strategies for numerous pest stink bug species in multiple crops in North and South America.[17,21,22,25,58] These qualitative studies of stink bugs have defined waveforms for each strategy of feeding, with definite differences in appearances for cell rupture feeding, sheath feeding in phloem, and sheath feeding in xylem. Interestingly, within feeding strategies, all species have rather similar waveform appearances.[21] Thus, although heteropteran waveforms look very different from those of sternorrhynchans, they are similarly stereotypical among species, subfamilies and families.[21]

4.2 Beyond plant sap-sucking insects to blood-feeding arthropods

Significant gaps in knowledge exist surrounding blood-feeding arthropod behavior, vertebrate host response to arthropod feeding, and transmission of associated arthropod-borne pathogens because investigations into these events and activities are greatly hampered by their occurrence under the surface of host tissues. Furthermore, no *in vitro* research methods currently exist that can replicate the complex intersection of the blood-feeding arthropod vector, host (host immune response), and pathogens. This lack of methods limits the study of pivotal behaviors and events that can inform novel mitigation strategies. Application of EPG to blood-feeding vectors offers transformative potential to reveal the otherwise-masked feeding behaviors of medically and economically significant arthropods, including mosquitoes and ticks.

EPG can be broadly applied across all types of blood-feeding arthropods despite dramatic differences in their anatomy, behavior, and feeding strategies, because in all cases, fluid flows from host to feeding arthropod, and thus electrical current can pass. Therefore, EPG facilitates ready comparisons across traditionally separate research fields. Mosquitoes and ticks are examples of two types of blood-feeding arthropods with unique anatomy and feeding strategies. The mosquito stylet bundle (fascicle) consists of six separate structures that function together to pierce skin. Liquid (saliva and host fluids) pass through two separate canals within the maxillae; thus, as with hemipteroids, salivation occurs independently and through a different canal than does ingestion of blood.[59] Mosquitoes are ephemeral feeders, feeding from either blood vessels or from blood lesions. Using EPG (Fig. 4), numerous waveform families and types were observed for *Aedes aegypti* (L.) feeding on humans.[24] EPG captured salivation on the surface of skin, (waveform J), insertion of stylets into skin (waveform K), and stylet penetration through tissue layers (waveform L) to locate a blood vessel. Upon insertion of the stylet tips into the vessel, blood ingestion (waveform M) ensues and may continue until repletion (Fig. 5), which usually occurs rapidly (within 3–7 min).[24]

By contrast, ticks are long-term (3–14 days), blood pool-feeding arthropods. Ticks rachet and embed their mouthparts into host skin and use a single channel formed by the embedded hypostome and chelicerae that mediate the flow of saliva from tick-to-host and blood from host-to-tick.[59,60] The blood-feeding lesion is created and maintained through a cornucopia of tick salivary

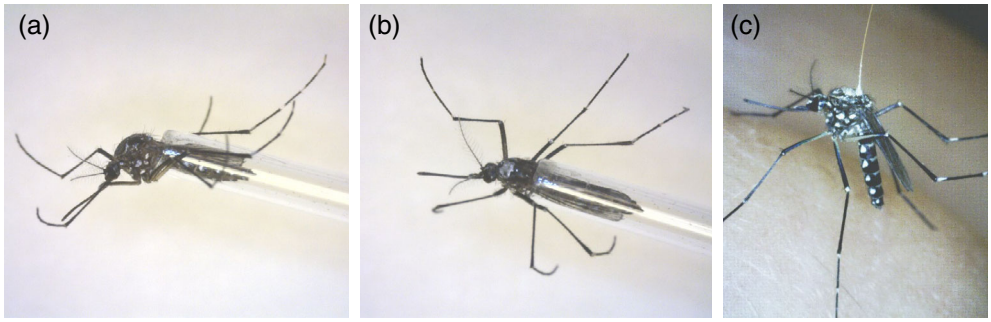

Figure 4. Steps for holding and attaching a wire tether to a mosquito. Similar steps are used for all other arthropods. (a) While viewing under a microscope, a mosquito is gently held at the tip of an aspirator by suction. (b) A dab of silver glue is painted onto its thorax. (c) A gold-wire loop is first dipped into silver glue, then rapidly affixed onto the dab on the thorax. Figure used with permission of Oxford University Press, from Wayadande, Backus, Noden, Ebert and Hillyer,[24] permission conveyed through Copyright Clearance Center, Inc.

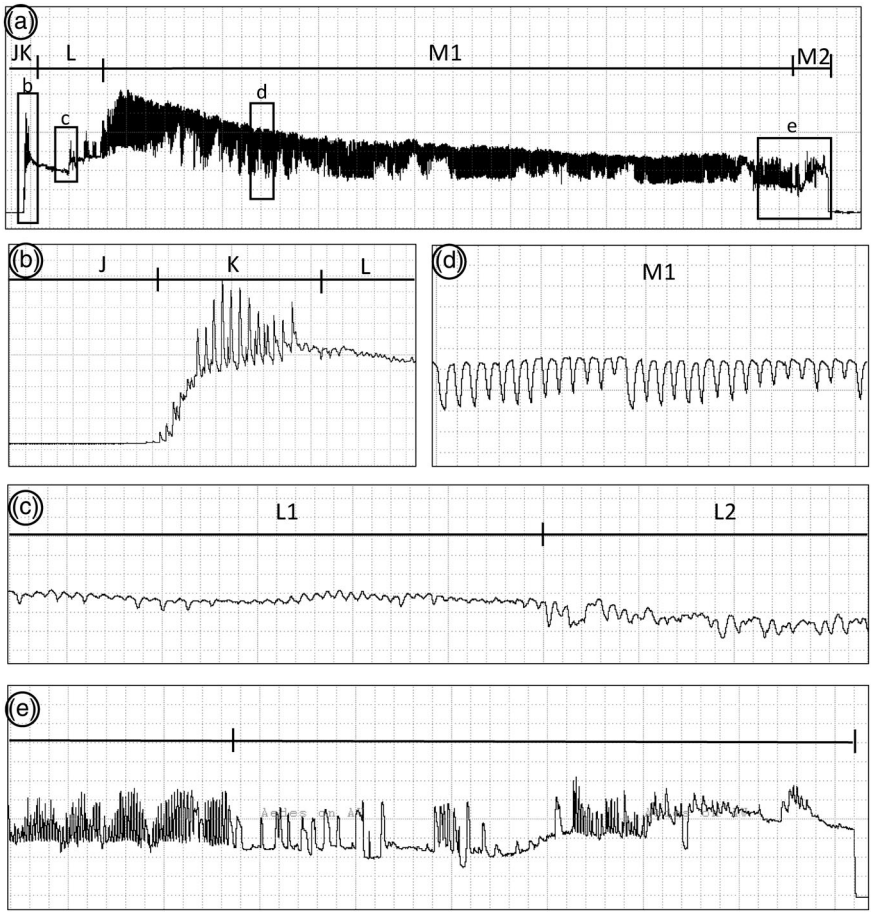

Figure 5. AC–DC EPG waveforms from mosquito *Aedes aegypti* on human hand. Recordings were done at Ri 10^7 Ω using applied signal of 150 mV AC to human hand, from reference.[24] (a) Overview of the entire mosquito stylet probe ('bite'). Family- and type-level names are along the top of the label bar. (b–e) Enlargements of boxes b–e in part (a). Labels similar to part (a). Time scales and Windaq gains were as follows: (a) 9.2 s/div, 8×; (b) 0.8 s/div, 8×; (c) 0.2 s/div, 8×; (d) 0.2 s/div, 64×; (e) 0.2 s/div, 8×. Figure used with permission of Oxford University Press, from Wayadande, Backus, Noden, Ebert, and Hilyer,[24] permission conveyed through Copyright Clearance Center, Inc.

proteins that recruit blood, prevent coagulation, and counter host immune recognition and responses at the feeding lesion. The highly orchestrated and temporally regulated processes of salivary secretion and blood ingestion are alternated during feeding, because the same mouthpart canal is used to convey both inward and outward flow of all fluids.[60,61] Initial EPG recordings of the Lone star tick, *Amblomma americanum*, at 20–48 h post attachment, reveal a progression of sequence-stereotypic waveforms (Reif KE and Backus EA, unpublished data).[35] Two waveform families have been identified: Aa (for *A. americanum*) 1 and 2 (Fig. 6a, b). Aa1 consisted of short, stereotypical episodes repeated every 10 s for 3–6 min in each cycle; Aa2 was a similarly structured (but longer and more complex) pattern that occurred at the end of a cycle of Aa1 episodes. Current inference is that both waveforms represent some type of salivation and/or sensory processing (Fig. 6a,c).

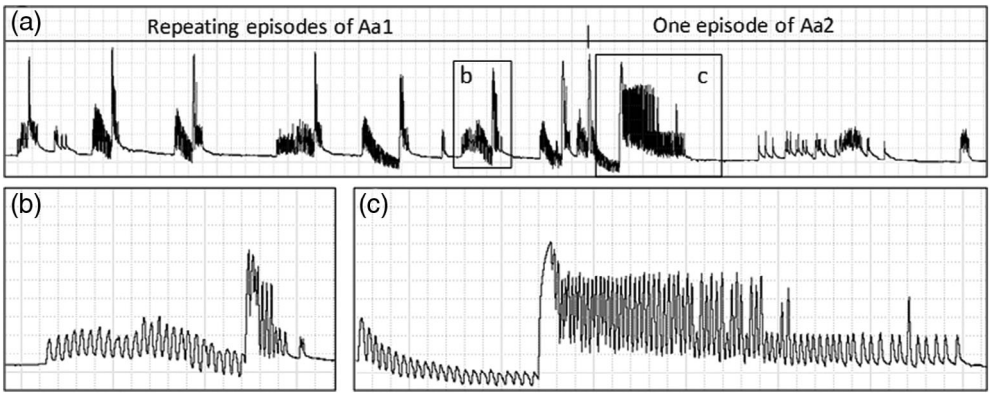

Figure 6. AC–DC EPG waveforms from tick *Amblyomma americanum* on calf. Recordings were done at Ri = 10^8 Ω, applied signal 350 mV AC to calf (Backus EA and Reif K, unpublished data). (a) Compressed overview of one event of Aa1, showing appearances of successive episodes. Labeled boxes contain waveform excerpts that are expanded in parts (b) and (c). (b) One episode of Aa1. (c) The early part of one non-repeating event/episode of Aa2. Time scale and Windaq gains were as follows: (a) 4.4 s/div, 64x; (b, c) 0.4 s/div., 128x.

EPG has great potential as a powerful tool to study fine-detail temporal activities at the blood-feeding vector/host–pathogen interface. Viewing mosquito stylet movement towards a blood vessel and ingestion activity was previously possible only by video-recording feeding mosquitoes as they probed animal tissues;[62] not even this level of information exists for ticks. EPG enables precise quantification of the specific activities that make up the behavioral sequence of an arthropod probing animal tissue. Thus, one can measure and compare differences in feeding behaviors between different species, strains, or engineered phenotypes. For example, EPG can be used to address questions regarding whether all mosquito genera probe in the same way (e.g. probe attempts, probe duration, waveform sequence), or, whether tick salivation patterns vary between different tick genera, life stages, or host types. EPG can also be used to investigate host responses to blood-feeding vectors, or discover differentially expressed host factors (e.g. volatiles, gustatory cues, immune response) that alter blood-feeding vector behavior. To illustrate, consider that EPG could be used to address how vector-feeding behavior changes when feeding on a susceptible, sensitized, or resistant host. Alternatively, EPG could identify the association between specific feeding behaviors/waveforms and specific host factors/responses.

EPG also would be a potentially useful tool to track parasite feeding behavior, as exemplified recently with a study of *Varroa* mite attacking honeybee.[44] EPG allowed long-term monitoring of *Varroa* mite feeding without direct human disturbance, and clarified for the first time the vexing nature of the mite–bee relationship as well as means of mitigation.[44]

4.3 Beyond feeding to movement and oviposition behaviors

Arthropods are dependent on their respective substrates not only for feeding, but also for survival, development, and/or reproduction.[63] Thus, the question that naturally develops is 'Why should we limit the potential of EPG to feeding studies?' The interactions summarized in the Introduction mediate arthropod adaptations to their substrate, establishing the underlying mechanistic links between behavior and physiology.

Walking, sheltering, and of course oviposition are important behaviors beyond feeding that are mediated by the arthropod's living substrate, which is also relevant for both inter- and intraspecific communication.[11,43,64] EPG can be used to detect egg-laying behavior of insect parasitoids; several distinctive waveforms were recorded during oviposition by the braconid *Euplectris comstockii* into larval *Tricoplusia ni* (Backus EA and Coudron T, unpublished data; personal communication). Application of EPG to parasitoid oviposition would not only provide a better understanding of the behaviors taking place, but also of the basis of decision-making and optimization by the parasitoid. In this case, the focus would be on egg-laying rather than on adult feeding behavior.

Another example of the potential value of EPG is its use to study egg-laying behavior of flies. Electronic monitoring of feeding in flies was previously achieved for *Drosophila* spp. fruit flies, but without segregating dabbing and ingesting events during the feeding phase.[65] Recent research with the AC–DC electropenetrograph provided higher resolution with the recording of waveforms for walking, grooming, and standing, in addition to the main events of feeding (segregating dabbing and ingesting) and egg-laying (segregating probing by the ovipositor and egg-laying *per se*) in the spotted wing drosophila, *Drosophila suzukii* (Fig. 7).[43] Sponging mouthparts of flies, despite their differences with the sucking mouthparts of hemipterans,[59,66] provided sufficient EPG waveforms to demonstrate recording of fly feeding behavior and recognition of the feeding dynamics taking place. Therefore, the success of EPG use for *D. suzukii* is not a surprise. More interesting, though, is the EPG recording of egg-laying behavior and preferences of *D. suzukii*,[43] which has a serrate ovipositor with enlarged bristles apparently allowing the insect to pierce the fruit skin to lay their eggs sub-superficially.[67] Nonetheless, the egg-laying waveform obtained from *D. suzukii* shows a steep spike inconsistent with the expectation for sawing movements, but consistent with a quick insertion and egg-laying, with the fly's serrated ovipositor used to just break the fruit exocarp.[43] Thus, EPG changed our understanding of oviposition behavior, supporting that the insertion of the terminal thorn bristle of the fly ovipositor is likely enough to break the (soft) fruit skin for egg-laying, better reflecting the dynamics of the process.

5 PERSPECTIVES FOR PEST MANAGEMENT

The close association of arthropods and their living substrate has relevance for arthropod life-histories and consequently for their management. Changes in substrate mediate arthropod colonization and injury, and such substrates are necessarily subjected

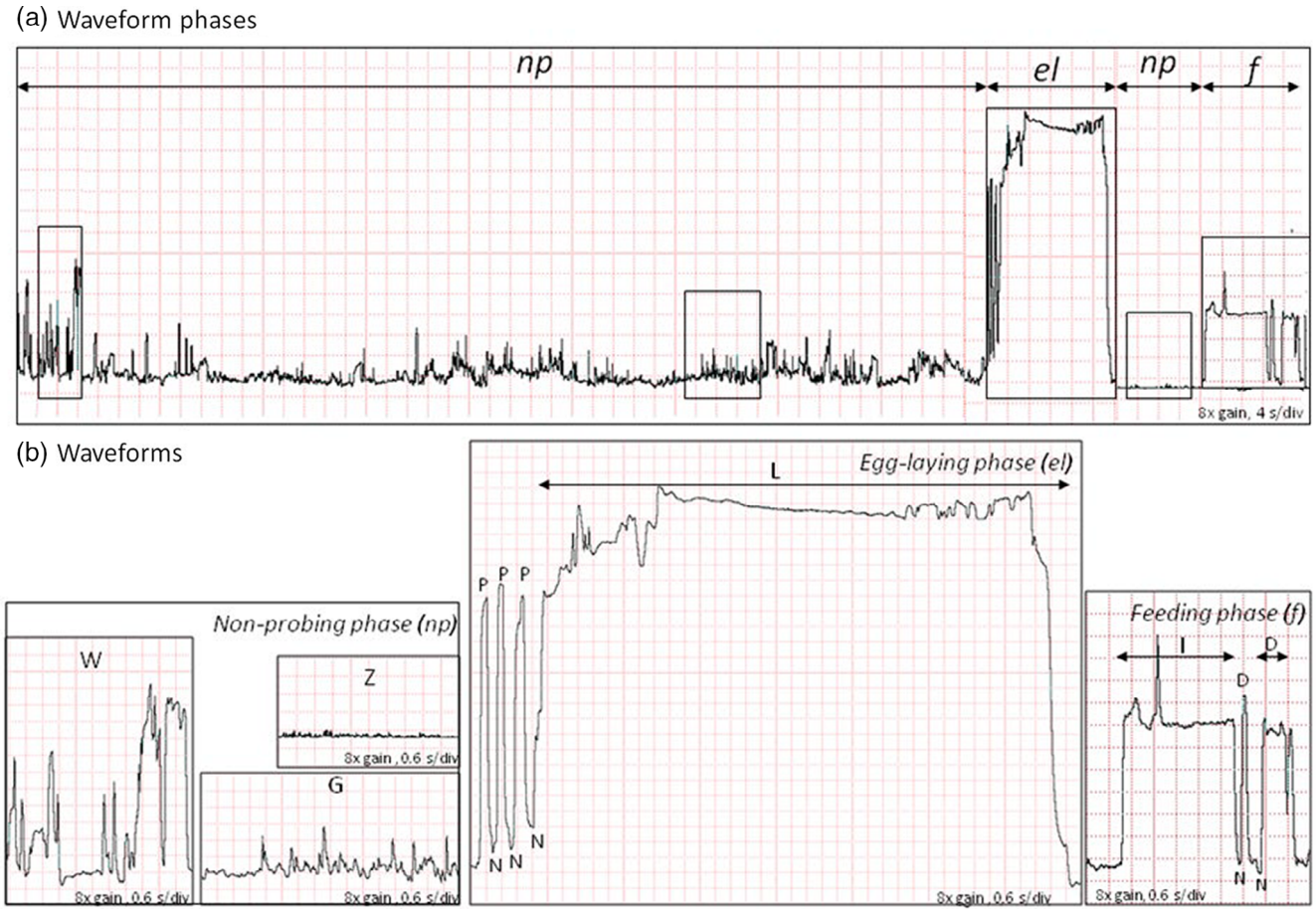

Figure 7. Overview of EPG waveforms of spotted wing drosophila, *Drosophila suzukii*, on strawberry. Ri = 10^9 Ω, applied signal 20 mV AC. (a) Waveform phases non-probing (*np*), feeding (*f*), and egg-laying (*el*). (b) Waveform types from the non-probing phase: resting (Z), grooming (G), and walking (W); from the feeding phase: dabbing (D), and ingesting (I); from the egg-laying phase: are probing (P), and egg-laying *per se* (L). Interruption of feeding and/or egg-laying is coded as N. Windaq gains and X-axis compressions are indicated in each recording. Figure used with permission of Springer Nature BV, from Guedes, Cervantes and Backus and Walse,[43] permission conveyed through Copyright Clearance Center, Inc.

to both environmental and anthropogenic influences.[11,32,43] Although environmental changes in substrate affect pest management, they do so indirectly. By contrast, anthropogenic influences on substrates are largely achieved by control methods directly applied against arthropod pest species. Thus, feeding damage and pathogen transmission are affected by host conditions, and control methods are usually designed to take advantage of the arthropod substrate to achieve control. As envisioned from its earliest years, the *raison d'être* of EPG is to perform qualitative studies to understand the nature of pest feeding and the damage it causes, then quantitative studies to aid in development of novel pest management tactics. We explore both goals, below.

5.1 Feeding damage and pathogen transmission by hemipterans

Basic feeding strategy studies of hemipterans via EPG (summarized above) combined with histological, biochemical, and molecular studies of plant hosts have had significant impacts on understanding the causes of damage by these pests. Again, only a short summary and a few examples can be provided here, but excellent reviews of plant pathogen transmission with multiple examples of EPG are referenced.[37,63]

Salivary sheath-feeders such as aphids primarily cause indirect feeding damage to crops as vectors of plant pathogens. Aphids can probe/penetrate their stylets in such a stealthy, intercellular manner that probed cells are not killed. Thus, aphids have evolved to be the perfect vectors of plant viruses, which require the cellular processes of living cells to replicate. Some viruses are nonpersistent and stylet-borne, meaning they remain in the vector for only a short time after acquisition, and are carried on a specific binding area, the acrostyle, at the stylet tips.[68] Acquisition and inoculation have been correlated with specific sub-phases of the potential drop (intracellular puncture) waveform,[69] or in certain species, a special type of potential drop (or pd), the phloem-pd, which represents penetration of a phloem sieve element or companion cell;[70,71] both types of intracellular punctures last only a few seconds. Late in the intracellular puncture, sub-phase II-3 takes up fluid that conveys virions to the acrostyle, causing acquisition. Early in the intracellular puncture, sub-phase II-1 represents salivation (or perhaps egestion[70]), which dislodges viruses from the acrostyle and out the stylet tips, causing inoculation into the phloem cell.[72] Persistent-circulative viruses are acquired during ingestion from phloem sieve elements (represented by waveform E2 by aphids, psyllids, and whiteflies).[72,73] The viruses then circulate in the hemolymph of the vector, invade salivary glands (and

often other organs), and are subsequently inoculated into healthy phloem sieve elements during phloem salivation (waveform E1 in aphids). Some circulative viruses can persist in the vector until their titer declines below an inoculatable level, because they do not propagate (replicate). Other viruses do replicate in the vector; thus, they can be inoculated for the rest of the vector's life.[68]

Most auchenorrhynchans also cause indirect crop damage by transmitting plant pathogens, because they are salivary sheath-feeders similar to aphids. However, the intracellular feeding tactic of leafhoppers mostly narrows transmission to phloem-limited pathogens. A few leafhoppers are virus vectors, such as the well-studied beet leafhopper, *Circulifer tenellus*, the vector of the persistent-circulative *Beet curly top virus*.[74,75] Again, acquisition from phloem sieve elements occurs during phloem sap ingestion (waveforms D2, D3 and D4), and inoculation occurs during phloem sap salivation (waveform D1).[52] Unlike the above examples, most leafhopper vectors also transmit phloem-limited mollicutes such as *Spiroplasma citri*, causative agent of citrus stubborn disease in citrus, carrot, and other crops,[77] as well as one of the so-far unculturable organisms classified by DNA fingerprinting as *Candidatus* (abbreviated *Cand.*) Phytoplasma asteris, aster yellow phytoplasma. Interestingly, both mollicutes are transmitted by *C. tenellus*.[78] Mixed infections can sometimes occur in vectors (although not technically demonstrated for *C. tenellus*). Thus, the same *C. tenellus* EPG waveforms play a similar role in transmission of mollicutes.

Another example of a leafhopper-transmitted virus is *Maize chlorotic dwarf virus* (MCDV), a phloem-limited, semipersistent virus that is foregut-borne and non-propagative in the vector. That is, it attaches to sites in the precibarium and cibarium (or functional foregut).[79,80] Consequently, MCDV is thought to be inoculated by egestion (termed extravasation in the cited studies), that is, ejection of fluid from the functional foregut, without swallowing. EPG studies demonstrated that vector and non-vector species differ in the appearances of their X wave, as well as number and length of X waves performed, thus supporting that the X wave represents egestion and inoculation of MCDV.[81]

Sharpshooter leafhoppers and adult spittlebugs (or froghoppers) (Aphrophoridae) often or exclusively ingest from xylem, where they acquire xylem-limited (-localized) bacteria such as *Xylella fastidiosa*, causative agent of Pierce's disease of grape, as well as variably named scorch diseases in other plants. *Xylella fastidiosa* is unique among plant pathogens in being non-circulative but propagative and semipersistent (in nymphs, but persistent in adults), because it colonizes the functional foregut of its sharpshooter vectors from which bacteria are inoculated. Thus, like MCDV, *X. fastidiosa* is foregut-borne and inoculated via egestion. Extensive research, recently reviewed,[9,10,82] has shown that bacterial cells are loosened by saliva 'swished' around in the functional foregut, then egested ('dribbled' or 'spit') out the stylet tips in a bolus of mixed plant fluid, saliva, and bacteria that is injected into xylem cells. Various stages of this complex inoculation process are represented by parts of the sharpshooter and spittlebug X wave, especially XN (Fig. 3)[9,10] also called Xe.[83]

Unlike the above examples, some hemipteran pests are economically important due to their direct feeding damage (without pathogen transmission) despite being sheath feeders. Cereal aphids such as greenbug, *Schizaphis graminum*, Russian wheat aphid, *Diuraphis noxia*, and sugarcane aphid, *Melanaphis sacchari*, cause red-to-yellow striping and other discolorations as well as physiological damage leading to reduced yield.[84] Similarly, the aforementioned brown planthopper is the number one pest on rice in all of Asia because it causes hopperburn, severe chlorosis and necrosis of leaves. In all of these cases, EPG has aided in identifying the mechanism of damage, which is the injection of phytotoxic watery saliva into phloem sieve elements during waveform E1 for the aphids and N4-a (possibly also N3, the X wave) in brown planthopper.[55,85] Biochemical and proteomic studies have identified salivary protein effectors of these reactions,[86–88] sometimes in combination with EPG.[89]

Leafhoppers in the subfamily Typhlocybinae also cause direct damage, but by a different mechanism; they feed by cell rupturing.[48] EPG was instrumental in identifying how their unique feeding causes two types of direct feeding damage, termed stippling (white spots on leaves, causing little economic damage) and hopperburn, a devastating problem in South America and Asia, where crops are stunted, chlorotic and have reduced foliage and seed yield.[90] Different tactics of cell rupturing cause different damage, and all are represented by different EPG waveforms.[48] All include some level of salivation.

Heteropterans such as plant bugs (Miridae) use cell rupture feeding, whereas stink bugs (Pentatomidae) use both cell rupture and sheath feeding. AC–DC EPG was effectively used to identify the cell rupture strategy, and how it causes cotton bud damage by *L. lineolaris*.[32,42] EPG of stink bugs, especially combined with plant histology, has shown damage to reproductive structures from cell rupturing *versus* less damage to stem vasculature from sheath feeding.[21]

Despite the ubiquitous role of saliva in both indirect and direct feeding damage, no EPG research to date can differentiate between salivation or ingestion free of viruses *versus* containing viruses.

5.2 Feeding damage and pathogen transmission by blood-feeding arthropods

Public and veterinary health as well as associated economies are impacted by blood-feeding arthropods through both direct and indirect means. Direct actions such as bites cause irritation, stress, and blood loss that can individually or collectively lead to significant reductions in livestock and human health and welfare, as well as production parameters in livestock. Indirect impacts of blood-feeding arthropods can include secondary infection of the bite site and, most importantly, pathogen transmission. Although EPG of blood-feeders is in its infancy, its potential is very great. EPG can be used to evaluate feeding activities of blood-feeders associated with pathogen transmission, including behavioral manipulation of vectors by their pathogens and timing of pathogen inoculation. For example, previous studies have suggested that *Plasmodium* parasites alter their mosquito vectors' host-seeking behaviors to favor dissemination. EPG could be used to precisely and quantitatively address how *Plasmodium*-infected mosquito behavior is altered.[91] The role of parasite metabolites, naturally occurring phagostimulants common to blood-feeders, and vector saliva components on feeding behaviors needs further, precise investigation. Pathogen inoculation to specific tissues can be determined by using EPG to record a competent vector during inoculation and then stopping feeding at key waveform intervals. Those and many more studies are made possible by EPG.

5.3 Host plant resistance

Arthropod–surface interactions and their EPG monitoring also have been used to study host plant preference, avoidance, and resistance as a tactic of pest management. Such EPG use is not

new, dating back to the 1990s, but usually focuses on sap-feeding insects such as whiteflies,[92] aphids,[93–95] and psyllids.[96] In all cases of EPG research, basic qualitative studies of behavior in relation to plant damage, summarized above, have enabled later quantitative studies to measure waveforms and compare hemipteran feeding on multiple host plants representing a spectrum of resistance. Sophisticated analytical techniques for such studies have been developed over the years to identify significant differences among waveform durations or numbers.[43,97,98] As in other sections, we have strived to find example from all three generations of EPG monitors, because all instruments made lasting contributions.

Host plant resistance was originally divided into three categories: antibiosis, nonpreference (later renamed antixenosis), and tolerance.[99] Today, antibiosis and antixenosis are combined (simply called resistance, with further subcategories) because they often overlap mechanistically.[100] Most EPG papers studying host plant resistance use the older, trichotomous framework. Resistance studies using EPG can be especially valuable for antixenosis. Such plants produce chemical compounds that change insect behavior through repellency before feeding, or deterrence after feeding begins. Antibiosis (caused by toxins), or a combination of antixenosis and antibiosis, can be detected as well, if wired insects are allowed long recording times (>10 h) or held after recording to determine subsequent mortality.[101] In other words, an insect-toxic plant may not immediately affect behavior, but ingestion of its toxins may affect later behavior or cause early death. One of the earliest EPG studies was the effect of sinigrin on aphids.[102] Among the best-studied systems are aphids on cereals with hydroxamic acids such as 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA).[103]

Many EPG studies have identified a common behavioral pattern exhibited by most sheath-feeding aphids and leafhoppers on antixenotic and/or antibiotic plants. Typically, sheath feeders like aphids spend short times locating their preferred ingestion cell type (usually a phloem sieve element) during behaviors specialized for searching and salivary sheath formation (termed pathway activities). Most of their probing time is spent performing ingestion from their preferred cell type during very long-duration ingestion events, resulting in long probes (many minutes to several hours). However, on resistant plants, the same species often reverse this process. Because the normal progression of feeding behaviors and stylet depths is stereotypical, reactions can be attributed to 'resistance factors' at certain locations in the plant identified from waveforms impacted by host plant resistance.[93–95] Thus, on resistant plants, probes can be short, usually aborted by insects that have difficulty finding their preferred ingestion cell. Pathway activities can be significantly longer as the insects spend protracted time in searching.[104,105] Phloem ingestion durations can be significantly shorter (depending on the location of the resistance factor), as noted in both AC[106] and DC[107,108] studies of effects of the Mi and Vat resistance gene in aphids[106–107,109–110] and in whiteflies.[108] Alternatively, ingestion can be deflected to xylem instead of phloem by starving, desiccated insects.[111] Some studies have used artificial diets to identify reduced ingestion due to lectins[112] that could be expressed in plants to confer transgenic resistance, or other compounds such as genistein[113] or hydroxamic acids (DIMBOA)[114] already found in resistant plants.

Although seldom studied using EPG, tolerance can sometimes also be detected by changes in pest behavior. An unusual example of this was seen with *Empoasca kraemeri* recorded on a spectrum of tolerant bean plants. A cell-rupture feeder, *E. kraemeri* is the number one pest on common beans in South America because its feeding causes hopperburn.[90] This direct feeding damage is proportional to a specific variant of the lacerate-and-sip tactic called pulsing laceration, usually performed in phloem tissues.[48] Statistical comparison of feeding on five bean genotypes [one susceptible (called check) genotype and four variously resistant genotypes] showed no differences in overall amount of feeding on any plants; insects spent almost all their time feeding. However, they could 'mix-and-match' among four feeding tactics and variants on different genotypes. Some so-called tolerant genotypes were actually antixenotic; they all stimulated a switch in behavior to decrease pulsing laceration compared with the check genotype, thus decreasing hopperburn. By contrast, a truly tolerant genotype stimulated increased pulsing laceration, but still exhibited less hopperburn and preserved bean yield by producing healing/compensatory plant responses.[115] The EPG results were compiled and summarized using multivariate statistics to develop a type of resistance index called a Stylet Penetration Index.[116] Results of this index could perfectly duplicate a simultaneously calculated resistance index using field measurements of bean yield from the same five genotypes.[48] Importantly, EPG could produce the Stylet Penetration Index results in about 6 weeks of work in a greenhouse and laboratory, while the field resistance index required over 6 months of effort in the field with intensive labor to grow, harvest, and count bean plants and beans.[48,116] A similar index could be developed for important hopperburning pests worldwide, such as *E. fabae* on alfalfa in North America,[117] *E. onukii* on tea in Asia,[118,119] and *N. lugens* on rice in Asia.[88] Development of indices for other hemipterans, both cell rupture and salivary sheath feeders, is a reasonable goal for EPG host plant resistance projects for the future.

5.4 EPG and chemical or other control methods

The original interest by early EPG researchers in aphid feeding and plant pathogen transmission was motivated by their intuition that interfering with either sap content or substrate surface may impair the feeding process and potentially the pathogen transmission process,[6,7] providing a new avenue for pest management research. Several AC monitor experiments studied the effects of systemic insecticides on aphids[6] and leafhoppers.[120] Similarly, plant water stress and fertilization can interfere with sap-feeding.[121] Both are amenable to high-resolution monitoring via EPG that may have consequences for pest management. Nonetheless, the use of systemic insecticides and miticides have more direct impact on sap-sucking insects, an effect that can be directly detected via EPG.

Neonicotinoids are the main insecticides targeted in modern EPG studies with sap-feeding insects. Regardless of the mode of application, e.g. seed treatment or soil drenching or other, insecticides usually extend non-probing behavior and/or enhance probing, and change sap-ingestion patterns (similar to behavioral patterns described above for host plant resistance, although the response can vary with pest species).[122,123] Recently, an EPG study showed that only imidacloprid (among a large array of compounds tested) could kill the Asian citrus psyllid, *Diaphorina citri*, before its stylets reached the phloem.[124] Thus, only imidacloprid could prevent inoculation of the phloem-limited bacterium *Cand.* Liberibacter asiaticus, the one causative agent (of three, worldwide) of huanglongbing (HLB) or citrus greening disease that is found in Asia and the Americas.[124] Changes in sap-ingestion behavior are not restricted to neonicotinoids but extend to other plant systemic insecticides, such as the diamide cyantraniliprole,

pymetrozine, and other contact insecticides, antifeedants, and the like.[125–128] The antifeedant and growth regulator azadirachtin was one of the earliest targets of EPG research in aphids and leafhoppers,[111,129] along with flonicamid and pymetrozine in aphids, leafhoppers, and psyllids.[130,131] The latter compound, pymetrozine, blocks the transient receptor potential vanilloid (TRPV) channels of chordotonal organs, thus directly and selectively interfering with feeding behavior of sap-feeding insects, leading to their starvation.[132,133] By contrast, flonicamid inhibits inward rectifier potassium (Kir) channels of epithelial and glandular systems, compromising salivary activity and fluid-feeding.[134,135]

Arthropod pest management impacts of EPG for contact insecticides include and go beyond sap-feeding insects, although other insects have been largely neglected. However, a recent research example will suffice – not only for insecticides, but also fungicides.[11,38] EPG of *D. suzukii* on strawberries treated with the insecticide spinetoram reveals impaired feeding and compromised adult longevity when in sublethal exposure, but even the fungicide fenhexamid exhibits mild effects impairing feeding of adult flies.[38] In addition to feeding impairment, egg-laying behavior is likely compromised by insecticide exposure as well, either via sap-feeding or contact exposure, a topic that deserves further attention. As exemplified in this study, EPG can be instrumental in recognizing the underlying mechanisms of behaviors beyond feeding. No doubt, EPG-based pesticide studies should be considered for other experimental systems of chewing, lapping, and sucking feeders from diverse, non-hemipteran orders. Although no studies of beetle feeding have been published, preliminary feeding waveforms recorded with an unknown flea beetle (Coleoptera: Chrysomelidae) (Backus EA, unpublished data) suggest that chewing feeders are highly amenable to EPG, due to its ability to discern both salivation and ingestion.

Diet suitability and EPG studies with phytophagous, non-hemipteran insects are also long overdue. *Drosophila suzukii* exhibits changes in feeding behavior when on artificial diet, ingesting less on artificial diet than on strawberries.[43] This finding illustrates limitations of diets for research compared with a natural, more preferred feeding substrate, despite the prevalent use of diets in laboratory research on *D. suzukii*.

Another area that may greatly benefit from EPG use is biological control because the technique allows high-resolution recording of the parasitism or parasitoidism. Although studies of parasitoid feeding and oviposition are mostly lacking, recent research using EPG to verify that *Varroa* mites feed on honeybees[44,136] suggests that such an area of study would be promising. The EPG-monitored mechanics of *Varroa* mite feeding on honeybee pupa indicate that the former is able to adjust its feeding apparatus and behavior to acquire the necessary amount of food.[136]

In addition to revealing oviposition behaviors of parasitoids, suggested above, EPG can also have an impact on biological control by examining feeding of prey insects subjected to predation. A unique paper[137] used EPG to examine inoculation-related behaviors of the leafhopper, *Psammotettis alienus* (which transmits the phloem-limited *Wheat dwarf virus*) with and without the presence of a spider predator, *Tibellus oblongus*. In the presence of the spider, leafhoppers reduced the duration of phloem salivation events, during which virus inoculation takes place. Phloem ingestion, when virus acquisition occurs, was delayed and occurred less often. Thus, presence of predators in an agroecosystem can possibly decrease the transmission of plant pathogens by local vectors.[137]

In similarly revolutionary ways, EPG has enormous potential to impact pest management of blood-feeding arthropods. Control of blood-feeding arthropods is largely accomplished through use of chemical compounds that kill and/or repel arthropods. Chemical control for mosquitos and ticks generally relies on using repellents or sprays in varying concentrations, such as diethyltoluamide (DEET), applied via topical lotions, spot-ons, or sprays in varying concentration. An array of chemicals, chemical formulations, and application options are available to protect humans, companion animals, and livestock. Recently, a class of systemically acting chemicals, the isoxazolines, was developed that offers systemic protection by rapidly killing blood-feeding arthropods upon bite/probe of the host.[91,138] EPG offers a novel approach to study disruption of blood-feeding arthropod behavior in response to chemical control measures. For example, EPG can be used to evaluate chemical repellency properties through measurements of landing and probing, and chemical disruption of feeding events and pathogen transmission through measurements of salivation and ingestion events and durations.

5.5 Transgenic crops and mosquitoes

Development and marketing of transgenic crop plants, such as *Bt* cotton, that express insecticidal proteins from the bacterium *Bacillus thuringiensis* (*Bt* proteins) has virtually eliminated lepidopteran and coleopteran pests in those crops, at least for now. Consequently, formerly secondary pests such as thrips and heteropterans have increased in importance in crops such as cotton, in recent years.[139–141] A case in point is the tarnished plant bug, *L. lineolaris*, which is a major pest on cotton in the southeastern USA because nymphal feeding causes damage to developing flower buds. Recently developed, transgenic cotton expressing the new *Bt* protein Cry51Aa2.834_16 was demonstrated to cause mortality of nymphal *L. lineolaris*.[139–141] EPG research showed that these *Bt* cotton plants were actually antixenotic because they were less palatable or preorally digestible to *L. lineolaris* nymphs.[33] Another EPG study showed the same *Bt* cotton also had antifeedant effects on thrips.[142] *Bt* proteins are produced in almost all tissues, yet are not systemic. Therefore, the feeding changes reported are surface-mediated effects and the question remains whether such effects extend toward egg-laying as well, which was not recorded in the above-cited studies.

The advent of transgenic mosquitos affords new opportunities in potentially similar ways, to study the effects of planned mutations upon feeding behavior and pathogen transmission. CRISPR Cas9 and other gene-editing strategies have enabled rapid generation of targeted alteration of mosquitos and other species. To date, major vector genera, including *Anopheles*, *Culex*, and *Aedes*, have been subjected to gene editing to reduce pathogen (*Plasmodium* and arbovirus) transmission and reduce fitness through a number of modification strategies, including impaired pathogen movement through salivary gland barriers.[143,144] For example, targeted salivary gland function in *Anopheles* revealed impaired ability to feed on mice,[145] but precise determination of stylet activities was not possible by visual observation. EPG analysis of transgenic mosquitoes may reveal differences in salivation time, increased/decreased searching for blood vessels, or insertion without activity, all of which may impact pathogen inoculation efficiency. Transgenesis may also have unintended consequences that could best be discovered by in-depth EPG analysis.[146]

6 CONCLUSION

EPG-obtained information has led to impressive gains in knowledge about and management of hemipteran pests, as well as their impacts on global agriculture. With new or improved developments in EPG technology, these benefits are being expanded and can be applied to any and all arthropod pest systems. We stand at the threshold of great possibilities in many formerly intractable systems, such as studies of blood-feeding arthropods. EPG should enable unprecedented investigations into the fine details of vector feeding, vector-host immunologic interactions, pathogen manipulation of vector feeding behaviors, and pathogen inoculation. Research questions and applications to other pest systems are limited only by the imagination and creativity of scientists around the world. EPG is ready for the challenge.

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CONFLICT OF INTEREST

E.A.B. co-invented the AC-DC electropenetrograph while an employee of the USDA, which filed a patent granted as US 8,004,292. As patent owner, the USDA has licensed the patent to Andrew M. Dowell, owner of EPG Technologies, Inc. By statute, E.A.B. is authorized to receive a small portion of royalty payments remitted to the USDA, if any. Also, with USDA permission and as part of her salaried position, E.A.B. is an advisor to the licensee to assist in instrument improvements.

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