Potential use of electrical penetration graph (EPG) technology for biosecurity incursion response decision making

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Abstract Sap-sucking insects pose a significant biosecurity risk as they can cause direct damage to plants and may also vector serious plant pathogens. However, there is a paucity of tools for rapidly estimating the host range of such insects during a biosecurity incursion. The Electrical Penetration Graph (EPG) technique has been developed over the past 50 years for analysing, quantifying, and comparing the feeding behaviours of both phloem and xylem sap-sucking insects. Electrically generated waveforms associated with insects' probing and feeding behaviours may allow a rapid assessment of the potential host range of invading sap sucking insects. This paper reviews the development and use of EPG, assesses potential for host-range testing of recent incursions, analyses when and how the EPG tool could be applied for response, and identifies seven points where EPG could aid biosecurity incursion response decision making. Three recent biosecurity responses could have benefited from EPG.

Keywords Host plant, host range, Electrical Penetration Graph technology, Biosecurity Response Knowledge Base, BRKB.

INTRODUCTION

Biosecurity agencies must rapidly assess the risk posed by invasive, phytophagous insects when they are first discovered in a new area or country. In New Zealand, the Ministry for Primary Industries (MPI) is responsible for the investigation and risk assessment of new incursions (Froud & Bullians 2010). The MPI then works in partnership with potentially affected industries to decide on response actions, which include eradication, long-term management or no action. An important aspect of this process is assessment of potential host plants of the new

insect (Burnip & Froud 2008; Burckhardt et al. 2014). There is a paucity of tools for rapidly estimating the host-range of hemipterans, commonly referred to as sucking insects (Martin 2008; Burckhardt et al. 2014). Hemipterans pose a significant biosecurity risk as they can cause direct damage to plants and may also vector serious plant pathogens (Nault 1997; Madden et al. 2000). The mouthparts of Hemiptera have been adapted for piercing and sucking host plant tissues. The mandibles and maxillae are modified as needle-like stylets. The stylet bundle consists

of two canals: one for secreting saliva and one for sucking sap out of the plant tissues. The stylet pathways in plant tissues can be intracellular, intercellular or a mixture of both and varies among insect groups depending on whether they are xylem or phloem feeders (Spiller et al. 1985; Tjallingii & Hogen Esch 1993).

The Electrical Penetration Graph (EPG) technique has been developed over the past 50 years and is now a vital tool for analysing, quantifying, and comparing the feeding behaviours of both phloem and xylem sap-sucking insects. There is potential for EPG to determine host-plant associations during biosecurity incursions to assess the environmental threat of an organism, identify which industries may be affected and to support response actions (Sandanayaka et al. 2013). However, to date, EPG has not been considered for host-plant determination for biosecurity incursions.

The host range of invasive insects may be already known from the international literature. However, when this is not available, host plants must be inferred by the plants the invasive insect was detected on or estimated from knowledge of related insects. Traditionally, the host range of poorly understood invasive insects has been ascertained by experimental assays. These assays expose insects to selected plants, quantify the extent of feeding and assess the physiological development and reproduction of the insect (Withers et al. 2011).

The objective of this paper is to explore the potential use of EPG for biosecurity risk assessment and response decisions. This was achieved, firstly, by undertaking a literature review of the technique and describing the process for its application and, secondly, by describing the key decision points in biosecurity risk assessment and response where the tool could be applied. An assessment of how EPG could have been utilised for recent insect incursions in New Zealand is made.

METHODS

Literature review

A literature survey was conducted to assess the

utility of EPG to rapidly identify host-plant associations of invasive sap-sucking insects.

Assessment of biosecurity decision points for EPG

Key decision points in biosecurity risk assessment and response where the tool could be applied were identified by searching the MPI online biosecurity response knowledge base (BRKB) (Ministry for Primary Industries 2010). The BRKB gives the policy for responding to risk organisms and details the processes, procedures and tools for biosecurity responses. Examples of how EPG could be used for each decision point were developed based on the BRKB guidelines. A flow diagram of the possible process for using EPG in a biosecurity response was developed based on the literature review and assessment of biosecurity decision points.

Case studies for EPG

A list of all insect responses in the last 10 years was provided by the MPI collated from their response database and from the risk analysis database. Each organism was assessed to determine whether there was evidence of EPG use in species in the same taxonomic family, which would indicate if EPG may have been suitable for the risk organism.

RESULTS

Literature review

Key papers relating to how EPG may be applied to biosecurity incursions were identified and summarised from 416 papers found from literature on insect feeding monitor and EPG between 1964 and 2016.

Hemiptera host-plant assessment

Host-plant acceptance by Hemiptera can be assessed in terms of food ingestion and/or reproduction (Powell et al. 2006). Before EPG, host selection and acceptance by sucking insects were measured by observations of the initial behaviour of an insect after alighting on a plant surface (i.e. duration of the first walk or proboscis contact period (Tjallingii 1976)). However, aphids (and probably most Hemiptera) do not show clear discrimination between host and

non-host plants until they have inserted their stylets (Powell et al. 2006) so initial alighting observations are not useful.

Host acceptance by sucking insects has been assessed by visually observing the behaviour of insects on selected plant species or quantifying their excretory droplets (Pathak et al. 1982). These methods are time consuming and also honeydew secretion could be partially controlled by environmental factors influencing the insect and its host (Dungan & Kelly 2003) so it is not a reliable method.

Electrical Penetration Graph (EPG) technique

The EPG technique was developed over 50 years ago and has been modified in a number of ways since then (McLean & Kinsey 1964; Tjallingii 1978; Backus & Bennett 2009). An EPG system consists of a monitor with two electronic components, a voltage source and an input resistor that are electrically connected to each other. A wire from the input of the monitor is attached to the insect using a special glue containing silver to make it conductive. A conductive object, such as a thick copper wire, from the output of monitor (plant electrode) is inserted into the plant tissue or the potting soil in which the plant is rooted. The EPG thus ‘electrifies’ the plant and measures changes in electrical resistance when an insect’s stylets probe different parts of a plant as part of an electric circuit. Fluctuations in voltage are caused by stylet penetration and positioning activities within the plant tissues (Tjallingii & Hogen Esch 1993), which change the electrical resistance over time, and are recorded as waveforms. The occurrence, sequence and duration of each stylet penetration event (such as time until the first probe, total probing time, duration of feeding (either phloem or xylem) and sustained ingestion) can be interpreted using various wavelength parameters (Prado & Tjallingii 1997).

Waveform correlations obtained from EPG have been used successfully to study feeding behaviour of many species from many groups of insects, including phloem feeders and a few xylem feeders (Table 1). The EPG technique has shown that the stylet of a phloem sap feeding insect may

briefly puncture cells in all tissues when it probes into a plant. However, the final decision on host-plant acceptance (i.e. feeding) is not made until the stylet enters the phloem or xylem (Sandanayaka & Backus 2008; Sandanayaka et al. 2013). The phloem cell is usually punctured toward the end of each probe. In non-host plants, stylet withdrawal from the phloem cell occurs rapidly, typically within three seconds, followed by stylet withdrawal from the plant (Powell et al. 2006).

Use of the EPG technique for host-range determination

The first EPG study on host range was reported by (McLean & Kinsey 1968) who compared probing behaviours of the pea aphid, *Acyrtosiphon pisum* on known host and non-host plants. Since then, the EPG technique has been applied to test host plant acceptance by many hemipterans (Table 1). Insects on hosts tend to have shorter salivation and longer ingestion periods. Insects on non-host plants typically have shorter first probes, longer pathway probing, longer non-probing periods and delayed or no phloem finding and subsequent ingestion (Calatayud et al. 1994; Lei et al. 1999; Liu & Takahashi 2000).

Holbrook (1980) screened 25 *Solanum* cultivars and one suspected non-host (*Pelargonium* cultivar) for acceptability to the green peach aphid *Myzus persicae*. The number of probes and the time spent in probing were indicators of relative acceptability in a 15–30 min recording period, and an “acceptability index” was worked out on the basis of those two EPG measurements on the test plant and the control plant. Girma et al. (1994) calculated a Potential Phloem Ingestion Index ($PPII = (PI/PPI) \times 100$) of Russian wheat aphid *Diuraphis noxia* on a range of plants. In that equation, PI is total time of ingestion from the phloem and PPI is the potential phloem ingestion or total recording time minus the time taken to perform the first committed phloem ingestion (>15 min ingestion). The PPII was found to be high in host plants and low in non-host plants. Tjallingii (1995) also reported that carefully selected EPG parameters could be used to identify the location of stylets in plant tissues to study the

Table 1 Examples of Electrical Penetration Graph (EPG) use across a range of hemipteran families and the EPG recording times used in host plant acceptance studies.

Insect family	Insect species	EPG recording period ^a (h)	Reference
Aphididae	<i>Schizaphis graminum</i>	3	Zúñiga et al. 1988
	<i>Rhopalosiphum padi</i>	3	Zúñiga et al. 1988
	<i>Rhopalosiphum padi</i>	8	Prado & Tjallingii 1997
	<i>Diuraphis noxia</i>	12	Girma et al. 1992
	<i>Myzus persicae</i>	10	Cole 1997
	<i>Aphis fabae</i>	8	Prado & Tjallingii 1997
Aphrophoridae	<i>Carystoterpa fingsen</i>	8–12	Sandanayaka & Page-Weir 2009; Sandanayaka et al. 2013
Aleyrodoidae	<i>Bemisia tabaci</i>	6	Liu et al. 2012
Cicadellidae	<i>Empoasca fabae</i>	2	Backus & Hunter 1989
	<i>Nephotettix virescens</i>	8	Liu & Takahashi 2000
	<i>Empoasca vitis</i>	5	Jin & Baoyu 2007
	<i>Homalodisca vitripennis</i>	20	Sandanayaka & Backus 2008
	<i>Orosius orientalis</i>	8	Trebicki et al. 2012
Coreidae	<i>Anasa tristis</i>	8	Bonjour et al. 1991
Delphacidae	<i>Nilaparvata lugens</i>	4	Hattori 2001
Miridae	<i>Lygus hesperus</i>	2	Backus et al. 2007
Pseudococcidae	<i>Phenacoccus manihoti</i>	12	Calatayud et al. 1994
	<i>Planococcus citri</i>	16	Costa Santa-Cecilia et al. 2013
Psyllidae	<i>Psylla pyricola</i>	7	Ullman & McLean 1988
Triozidae	<i>Bactericera cockerelli</i>	12	Sandanayaka et al. 2014

^a The EPG recording period is defined as the total duration (h) that the insect was monitored for feeding.

plant factors responsible for host acceptance.

Similarities in EPG waveforms from many species have allowed a standardised labelling system to be developed that represents key stylet penetration activities, such as intracellular punctures, phloem salivation and phloem ingestion (Reese et al. 2000). The standard labelling system has allowed EPG to reveal comparative differences in feeding behaviour shown by insects when feeding on different host plants and could be applied to ascertaining the host range of hemipterans in biosecurity responses (Sandanyaka et al. 2013). Therefore, these authors proposed that EPG may allow a rapid assessment

of the potential host range of invading sap-sucking insects.

Using the EPG technique for biosecurity response decision making

Successful operation of an EPG requires a degree of skill and experience as does interpretation of the output data (the waveforms generated by monitoring the feeding behaviour of the insects). However, this equipment is relatively cheap to operate in comparison with setting up alternative infrastructure for host-plant testing of chewing insects (Suckling et al. 2014). For use in a biosecurity response, the stylet penetration of

eight insects could be monitored simultaneously using a Giga-8 DC-EPG system (WF Tjallingii, Wageningen, The Netherlands). The data from the EPG monitor are acquired and stored using Stylet+d software (EPG Systems, Wageningen, The Netherlands). Faraday cages are used in all cases to reduce electrical interference. Despite the extensive literature on the EPG technique, there are no examples where host plant assessment has been undertaken using EPG during a biosecurity incursion. In New Zealand, EPG was applied following a biosecurity response for host plant assessment of *Bactericera cockerelli* (Tomato potato psyllid) but only after the decision was made not to eradicate this organism and the horticultural industry began long-term management of this new species (Sandanayaka et al. 2011).

A flow diagram of how the EPG technique would be applied during biosecurity responses is given in Figure 1. The use of EPG can be very flexible depending on the host range under examination. For example, a comparison test between a known host plant, and the suspect host can be conducted with as little as two insects within a 2-day period for a single host that an industry has concerns about (W.R.M. Sandanayaka, unpublished data). Ideally, replicates of 10 insects per host plant would be used to increase the confidence in the results (Sandanayaka et al. 2013). The one EPG unit currently available at Plant & Food Research, New Zealand enables 10 replicates of up to eight host plants to be screened over a 10-day period. Access to additional EPG units could be arranged within two weeks at a current (May 2017) cost of 4250 EUR (\$6450 NZD) per Giga-8 DC-EPG monitor (EPG Systems EU). In addition, the cost for gold wire and conductive silver paint consumables will be around 200 EUR (\$303 NZD).

A thorough understanding of “time to first probe”, “duration of first probe” “total probing time” “time to first phloem /xylem ingestion from the beginning of the recordings”, “time to phloem /xylem ingestion from the beginning of that probe”, “total duration of phloem/xylem ingestion” and “Potential Phloem/

Xylem Ingestion Index” in relation to the stylet penetration activities of an insect is necessary to assess host plant acceptance. For example, the information in Table 1 indicates that sufficient data to differentiate host and non-host plants can be obtained within a 3-hour recording period for some aphid species (Zúñiga et al. 1988). However, between 8 and 12 h of recording is required to demonstrate feeding in most tested aphid species (Girma et al. 1992; Cole 1997; Prado & Tjallingii 1997). Mealybugs spend a long time probing before starting phloem ingestion so a 12–16 h recording period is required to assess feeding for these species (Table 1). Of note, is that a recording periods range from 2–20 h for several Cicadellidae species were required to demonstrate adequate feeding. Therefore several preliminary tests may be required to determine the optimal recording period for species in this family. The information in Table 1 can be applied as an initial recording period for host testing as part of a biosecurity response. However, a minimum 10-hour recording period on a known host should be used to determine the optimal recording period of a novel insect species under a biosecurity incursion. The initial recordings will show the time to the first phloem or xylem ingestion. Plant acceptance will indicated by “time to first sustained phloem ingestion/xylem ingestion (>10 min ingestion)”, “total duration of phloem/xylem ingestion” and “Potential Phloem/Xylem Ingestion Index” (Sandanayaka & Backus 2008). Recording these EPG parameters for a known host will assist with determining the required recording period for the particular insect species on a range of potential host plants (known and unknown). Previous studies using EPG have shown that insects on non-host plants typically have shorter first probes, longer pathway probing, longer non-probing periods, and no, or reduced, feeding (by withdrawing stylets from the phloem/xylem cells) than on host plants (Sandanayaka et al. 2013). Therefore, these parameters can be used to determine the EPG test outcomes of host, suspected host and non-host in a biosecurity response.

Figure 1 Process diagram of the use of EPG within a biosecurity incursion response.

^a Adults are the preferred live stage for testing as they are more robust; however, for some larger hemipteran insects immature life stages could be used. ^b Shoots must be kept fresh during the experiment to avoid blockage of the sieve elements, which could affect feeding. ^c Changes from an initial recording period of 10 hours would be decided based on recommendations in Table 1 for specific species or families. ^d The Giga 8 EPG-DC monitor has eight channels so can monitor eight insects on eight plants for each recording period but more units could be used.

Using EPG as a tool to assist biosecurity decision making

There are several key decision points in a biosecurity incursion response (Ministry for Primary Industries 2011a). The first stage of a response is the Investigation Phase. In this phase, the risk is assessed to determine if an incursion has occurred, and a rapid risk assessment is made to determine if a response is required (Froud & Bullians 2010; Ministry for Primary Industries 2014). Biosecurity response decisions are, as of 1 July 2017, made in partnership with affected industries where a Government Industry Agreement exists.

A search of the Ministry for Primary Industries BRKB (Ministry for Primary Industries 2011a) found seven decision points where EPG may be able to assist with decision making in a biosecurity incursion, and examples of use are given in Table 2. Under existing response systems, a differential host range list (Figure 1) would be developed by the intelligence team as part of an organism science report. Such lists are compiled using data from literature reviews, risk analysis reports, scientific experts and technical advisory groups (Ministry for Primary Industries 2011b, 2012a).

Table 2 Biosecurity decision points where there is potential to assist decision making by using the EPG technique to understand the host range of hemipteran incursions.

Biosecurity Response phase	Decision point	Reference	Potential for EPG to inform decision?	Example of use
Investigation	Does a biosecurity risk remain?	Ministry for Primary Industries (2013a)	Yes	EPG use to ascertain risk organism threat to economic or environmental values where significant uncertainty exists around the host range. One confirmed host must be known.
Investigation	Determine if response recommended?	Ministry for Primary Industries (2012b)	No	Decision agreed between Incursion Investigation and Response teams. Unknown host range may initiate a response.
Transition to Response Phase	Under the Government Industry Agreements (GIA) partnership programme, who should be involved in a decision to respond?	Pascoe (2016)	Yes	Under GIA, affected industries become involved in decision making and funding a response. EPG use can determine which industries will be affected.
Response Phase	What is the host range? (Organism science report)	Ministry for Primary Industries (2012a)	No	Information on the known and suspected host range. Used to inform the differential host plant list for an EPG project.

Response phase	What is the host range? (Science and Research project)	Ministry for Primary Industries (2011c)	Yes	Where host range is unknown or ambiguous, Intelligence team will request a research project. EPG would clarify host range for operational plan development (for surveillance, tracing, movement control and organism management).
Response phase	What is the host range for movement control?	Ministry for Primary Industries (2013b)	Yes	EPG informs hosts in the movement control operational plans to prohibit movement within the incursion zone.
Response phase	What is the host range for surveillance and tracing?	Ministry for Primary Industries (2013c)	Yes	EPG informs hosts for the surveillance and tracing operational plan to target surveillance and identify movements of host plants for forward and backward tracing and surveillance visits.
Response phase	What is the host range for organism management?	Ministry for Primary Industries (2012c)	Yes	EPG informs hosts for the organism management operational plan to target eradication actions.
Response phase	How does the host range affect the costs and benefits of response options for eradication or no action?	Ministry for Primary Industries (2011d); Ministry of Agriculture and Forestry (2003)	Yes	EPG informs the assessment of response options. Host range information enables policy advisors to develop cost benefit analyses for decisions on eradication, no further action or transition to long-term management.

Case studies for EPG

Details for 19 biosecurity incursions of hemipteran insects, detected in New Zealand for the first time between 2006 and 2016, were provided by the MPI. (Table 3). Of these, four

have not established in New Zealand and the remaining 15 have established (Table 3). Of these 19 species, evidence of use of the EPG technique within the same taxonomic family exists for 14 (74%) of them.

Table 3 Summary of biosecurity incursions of Hemiptera insects, detected in New Zealand for the first time between 2006 and 2016 (Berry and Gill, pers. comm.) including establishment status and whether EPG has proven effective for use in members of the same taxonomic family.

Scientific Name	Common name if given	Family	Year	Establishment	EPG used in family
<i>Bactericera cockerelli</i>	Tomato potato psyllid	Psyllidae	2006	Yes	Yes
<i>Bemisia tabaci</i> (Biotype Q)	Sweet potato white fly	Aleyrodidae	2006	Yes	Yes
<i>Planococcus citri</i>	Citrus mealybug	Pseudococcidae	2007	Yes	Yes
<i>Rhopalosiphum</i> sp.	-	Aphididae	2008	Yes	Yes
<i>Rhopalomyzus</i> sp.	-	Aphididae	2008	No	Yes
<i>Antonina socialis</i>	Cottony bamboo mealybug	Pseudococcidae	2008	No	Yes
<i>Carulaspis minima</i>	Bermuda cedar scale	Diaspididae	2009	Yes	No
<i>Melanaphis</i> sp.	-	Aphididae	2010	No	Yes
<i>Illinoia lambersi</i>	Rhododendron aphid	Aphididae	2010	Yes	Yes
<i>Acizzia solanicola</i>	-	Psyllidae	2012	Yes	Yes
<i>Thaumastocoris peregrinus</i>	Bronze bug	Thaumastocoridae	2012	Yes	No
<i>Neopinnaspis harperi</i>	Harper scale	Diaspididae	2012	Yes	No
<i>Tuberolachnus salignus</i>	Giant willow aphid	Aphididae	2014	Yes	Yes
<i>Myzocallis punctata</i>	Oak aphid	Aphididae	2014	Yes	Yes
<i>Phellopsylla formicosa</i>	Psyllid on eucalyptus	Psyllidae	2014	Yes	Yes
<i>Erthesina fullo</i>	Yellow spotted stink bug	Pentatomidae	2014	No	Yes
<i>Eucalymnatus tessellatus</i>	Tessellated scale	Coccidae	2015	Yes	No
<i>Chrysomphalus</i> sp. cf. <i>pinnulifer</i>	-	Diaspididae	2015	Yes	No
<i>Charagochilus</i> sp.	-	Miridae	2016	Yes	Yes

DISCUSSION

Assessment of the MPI biosecurity response knowledge base showed that the EPG technique could be used to inform response decisions at seven separate points. A key point early in a response is to use EPG to identify hosts that are important to specific industry groups. The EPG technique could also be used to identify hosts as part of the development of surveillance, tracing, movement control and organism management operational plans. In addition, the knowledge of which hosts may be affected (hosts and suspect hosts) is important in the development of a cost-benefit analysis of response options for eradication decisions. These points are supported by Burnip and Froud (2008) who showed that 33% of science inputs requested by the MPI were related to host information required to support the development of operational plans and assist containment and eradication options.

Over the last 10 years there have been 19 incursions of hemipteran insects in New Zealand (Table 3). Several of these have since become important economically damaging pests, e.g. (*Bactericera cockerelli* (tomato potato psyllid) (Teulon et al. 2009) and *Tuberolachnus salignus* (giant willow aphid) (Sopow et al. 2017) or minor pests *Acizzia solanicola* (Ministry for Primary Industries 2012d). The current review has identified literature relating to the efficacy of EPG in related insect species (Table 3) and indicated that EPG would have been suitable for use on 74% of these families. Every incursion is different and host range is just one of many factors that influences an eradication decision. However, with an average of two new incursions of sucking insects per year, it is likely that EPG could provide host range information to inform future response and eradication decisions in some species. The value of EPG to biosecurity authorities in the immediate aftermath of an incursion depends very much on the species, the extent to which its hosts are already known from overseas studies, and, perhaps, the feasibility of an eradication response.

To date, responses to incursions from sucking insect species have not benefited from host range information derived from using EPG.

However, the results of this study highlight the potential of, and describe the process for, use of EPG in future incursion events to assist in specific response decisions. There is evidence of EPG being used within the same taxonomic family as the three hemipteran incursions that have become important in New Zealand in the last decade. The EPG technique has subsequently been used to assess the host range and the vector ecology of one of these, the tomato potato psyllid (Sandanayaka et al. 2011). The giant willow aphid has been recorded on apples and pears in addition to willow (Sopow et al. 2017), and EPG could have usefully been used to determine if these important crops are feeding or shelter hosts. Similarly, the MPI requested solanaceous crop growers to report on the host range of *Acizzia solanicola* to inform their response (Ministry for Primary Industries 2012d) but EPG could have usefully been used to determine the host range.

The development of EPG over the past 50 years has resulted in a highly sensitive technique that can rapidly elucidate and quantify the feeding behaviours of sap-sucking insects. It is effective for both phloem and xylem feeders, and has been used to quantify and compare the responses of many different insects to a broad range of known host and known non-host plants. The EPG technique distinguishes between feeding behaviours that lead to acceptance or non-acceptance of a plant species based on the different durations of EPG waveforms for hosts and non-hosts. Waveform characteristics and interpretations have been validated across more than 10 different hemipteran families.

Limited examples of the optimal measurement period for some hemipteran families exist in the literature and there was variability in stylet penetration activities between species in the same family (Sandanayaka & Hale 2003). However, the optimal period of EPG recordings can be informed by initial tests on a known host plant over 1 to 2 days if prior knowledge of the insect and the test plant are not available. Ten hours initial recording period is recommended for testing any novel insect species on EPG (W.R.M. Sandanayaka et al. unpublished data).

An important limitation for use of EPG is that 'host acceptance' or 'host-rejection' by sucking insects may not be absolute. In nature, there may be a continuum between a 'host' and a 'non-host' that encompasses both 'good' and 'poor' hosts. In addition, a 'good' host species may become a 'poor' one (or even a non-host) within the course of a season as plant chemical defences, nutrition or environmental factors change.

In biosecurity incursions, decisions on host range are often targeted at the maximum host range to assist with movement controls of risk plants, surveillance activities and potential phytosanitary issues. Therefore, the determination of breeding hosts as opposed to feeding hosts is less important.

For EPG, some concern has been voiced that the gold wire attached to a test insect affects its behaviour and limits the 'reality' of the results (Prado & Tjallingii 1999). Results differ on whether the wire may or may not affect the feeding behaviour, depending on the species, individual insects, and the skill of the EPG researcher (Sandanayaka & Page-Weir 2009). The fact that wiring prevents an insect from escaping from an unwanted plant may result in eventual feeding on a non-host. However, the wiring constraint has negligible effect on comparative feeding parameters on different plants, whether hosts or non-hosts.

The EPG technique can provide rapid insights into the potential host range and food preferences of an insect that might not occur within the current distribution of the pest. This technique can be applied to other studies besides incursion response such as the transition to a long-term management programme of a new pest or measuring the rate of adaptation of invasive insects to existing or novel host plants in its new, expanding geographical range (Lazarotto et al. 2011). In fact, EPG has been widely used to assist with screening resistance to pests in crop breeding programmes (Dixon et al. 1990; Webster et al. 1993; Jiang et al. 2001; Alvarez et al. 2006). Another potential use of EPG is for biosecurity preparedness. For example, studies could be conducted on close relatives of high risk invasive organisms.

The interactions among pathogens, insect vectors and their host plants have been studied

extensively using EPG and this may be a wider application of EPG during biosecurity incursions. It can be used to reveal the efficiency of pathogen transmission and the minimum plant-access period required for disease transmission (Jiang et al. 2000; Moreno et al. 2005; Boquel et al. 2011; Sandanayaka 2014).

This review has summarised the inputs required, measurement, interpretation and outcomes of using the EPG technique for biosecurity incursions. Host-range outcomes can be provided within 2 to 14 days, depending on the biosecurity question and there is also potential to rapidly scale-up the volume of host-range testing for a large-scale biosecurity response at a reasonable cost.

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