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Choice of Tethering Material Influences the Magnitude and Significance of Treatment Effects in Whitefly Electrical Penetration Graph Recordings

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Abstract

The electrical penetration graphing or electropenetrography (EPG) technique is essential for understanding interactions of hemipteran insects with their host plants. Typically, 10–12.5 μm diameter gold wire is used as the tethering material in EPG studies. This wire was originally chosen based on suitability for aphids, but application of the EPG technique to other insects necessitates testing of alternative tethering materials that permit natural foraging and probing behavior. Whiteflies are one group for which EPG studies are increasing, with most researchers using 10 or 12.5 μm diameter gold wire even though these insects are smaller than aphids and very different in mobility. However, 2.5 μm diameter Wollaston process platinum wire has been used for a subset of EPG studies and seems to permit more natural movement and feeding behaviors. Here, we compared EPG variables derived from recordings of the sweet potato whitefly (*Bemisia tabaci*) tethered with 12.5 μm diameter gold wire or 2.5 μm diameter platinum wire. On a suitable host, gold-tethered whiteflies had reduced phloem phases, which are indicative of host plant acceptance, compared to platinum-tethered whiteflies. When we included a treatment known to reduce plant quality (methyl jasmonate application), platinum-tethered whiteflies exhibited expected reductions in EPG variables related to host acceptance, while gold-tethered whiteflies either had no response, or the opposite response. Our results indicate that tethering material strongly influences the outcome of EPG experiments, with important consequences for evaluations of host plant resistance, putative instances of plant virus manipulation, and feeding variables associated with virus transmission.

Keywords Electrical penetration graph · host plant resistance · probing behavior · potential drop · phloem ingestion · insect tethering

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Introduction

Unlike chewing herbivores, piercing-sucking insects ingest nutrients and transmit (i.e., acquire, retain and inoculate) plant pathogens during probing behaviors that are not directly observable. To study the salivation and ingestion activities of piercing-sucking insects, and ultimately understand the mechanisms of plant pathogen acquisition and inoculation, McLean and Kinsey (1964) developed the first equipment and protocols for electronic monitoring of insect feeding, in the 1960s. Their monitor and subsequently developed systems (termed electrical penetration graph [EPG] (Tjallingii 1988), or electropenetrometry [also EPG] (Backus and Bennett 2009)) enabled quantitative measurements of piercing-sucking insect stylet activities and positions in the plant tissues. During recordings, an insect and a plant are made part of an electrical circuit by connecting an “insect” electrode to the pronotum using a fine metal wire (2.5 to 25 μm diameter wires) using conductive glue, then inserting a separate “plant” electrode in the soil or other plant substrate. As soon as the insect stylets penetrate the plant tissues, the electrical circuit is completed, and a fluctuating voltage signal can be monitored and recorded. The voltage fluctuations of the signal form distinct patterns (named waveforms), which have been described with experimental correlations of probing activities (electrical waveforms) and histological localization of the stylet tips in the plant tissues (McLean and Kinsey 1967; Janssen et al. 1989). Aphids served as the initial models for EPG studies and the basis of the first waveform characterizations (McLean and Kinsey 1967; Tjallingii 1978). As a result, the techniques for use of EPG hardware, including development of insect tethering protocols, choice of wiring materials, and choice of wire diameters, were innovated and propagated among researchers with aphids in mind.

Today, thanks in part to availability of commercial EPG systems, the popularity of this technique has grown along with the number of insect species to which it has been applied. There are now studies describing waveforms produced by other insects in the suborder Sternorrhyncha (whiteflies, psyllids, scale insects, and phylloxera) (Janssen et al. 1989; Calatayud et al. 1994; Bonani et al. 2010), as well as some Auchenorrhyncha (cicadas, leafhoppers, treehoppers, planthoppers, and spittlebugs) (Almeida and Backus 2004; Jin et al. 2012; Cornara et al. 2018), and representative Heteroptera (Backus et al. 2013; Cervantes et al. 2017; Lucini and Panizzi 2018). The transfer of the EPG techniques from the aphid model to new insects is challenging because the “wiring” or “attachment” step must be adapted to ensure recording of natural behaviors and prevent introduction of tethering artifacts. For example, Lucini and Panizzi (2016) had to modify the aphid wiring technique for three stink bug species that have a large body size, and therefore perform vigorous movements compared to other smaller piercing-sucking insects commonly studied with EPG. Modifications included use of thicker gold wire and gentle sanding of the bugs’ thick cuticles with dental sandpaper. Recent work has also demonstrated that the type of adhesive (silver paint vs. silver glue) used to attach the wire to the insect can affect the appearance of waveforms (Cervantes and Backus 2018). Even with aphids, (Tjallingii 1986) showed that the wire attachment may cause reductions in fecundity and longevity and potentially contribute to behavioral differences between insects on host and non-host plants. Negative effects due to tethering were also detected for the aphid *Nasonia ribisnigri*, for which wired individuals showed less probing time than free ones due mainly to locomotion restraints (Helden 1995).

Despite the importance of proper tethering for preserving natural behavior and recording biologically meaningful waveforms, comparative studies of tethering protocols have not been a major focus of efforts to expand use of the EPG technique to new insect groups. Due to their global importance as virus vectors (Navas-Castillo et al. 2011), the Aleyrodidae (whiteflies) was the third family outside the Aphididae (after Cicadellidae and Delphacidae) to be studied using EPG (Backus 1994), with the first description of waveforms for *Trialeurodes vaporariorum* performed in the late 1980's by Janssen et al. (1989). But it is only recently that the EPG technique has become a routine tool for studying whitefly feeding behavior and virus transmission, with more than 50% of all whitefly EPG studies appearing in the literature after 2011 (Table 1). Nearly all of these studies employed a variation of the “aphid standard” protocol (described in detail in Cervantes and Backus 2018) using gold wire as the conductive tethering line (Fig. 1. b and d). In the case of whiteflies, most of which are highly mobile and only 1–2 mm long, researchers are constrained to use the minimum gold wire sizes available (10 or 12.5 μm diameter) to achieve any usable EPG recordings (Lei et al. 1997). However, in 1994, Walker and Perring introduced an alternative material - 2.5 μm diameter platinum wire (Fig. 1. a and c) - that is both thinner and more flexible than gold wire (Walker and Perring 1994). The flexibility of platinum wire permits more natural walking and foraging movements by this highly mobile insect, and the thinner diameter results in a reduced “tethering footprint” on the pronotum, which is much smaller in size than that of aphids.

Despite the fact that the majority (31/41–75.6%) of whitefly EPG studies were performed well after the recommendation to use the platinum wire tethering protocol (Walker and Janssen 2000), we found that 26/31 published studies still used 10–12.5 μm diameter gold wire as the tethering material (Table 1). Additionally, among

Table 1 Historic and descriptions of whitefly EPG studies

Wires	Thickness (μm)	Species	Stages	Source
Gold	10	<i>T. vaporariorum</i>	Nymph	(Lei et al. 1996)
			Adults	(Lei et al. 1997, 1998, 1999, 2001)
	12.5	<i>T. vaporariorum</i>	Adults	(Janssen et al. 1989; Tosh and Brogan 2014; McDaniel et al. 2016)
	10	<i>B. tabaci</i>	Adults	(Jiang et al. 1999, 2000, 2001; Seo et al. 2007; Yang et al. 2009; Yin et al. 2010; He et al. 2013; Shao-hua et al. 2015)
	12.5	<i>B. tabaci</i>	Adults	(Rodríguez-López et al. 2011, 2012; Liu et al. 2012, 2013a, b, 2017; Moreno-Delafuente et al. 2013; Jiao et al. 2014, 2018; Civolani et al. 2014; Ning et al. 2015; He et al. 2015; Zhang et al. 2016; Lu et al. 2017; Maluta et al. 2017, 2018)
	18	<i>A. proleptella</i>	Adults	(Broekgaarden et al. 2012)
Platinum	2.5	<i>B. tabaci</i>	Nymph	(Jiang and Walker 2003, 2007)
			Adults	(Walker and Perring 1994; Johnson and Walker 1999; Walker and Janssen 2000; Jiang and Walker 2001; Johnson et al. 2002; Zhou et al. 2017)

Fig. 1 **a** Platinum wire (2.5 μm diameter); **b** Gold wire (12.5 μm diameter); **c** Female whitefly tethered with platinum wire and **d**) Female whitefly tethered with gold wire (Photos by Ian M. Wright)

those studies using 2.5 μm platinum wire, quantitative data are not available on the frequencies and durations of various stylet activities because platinum wire studies focused on waveform descriptions rather than replicated experimental sessions (Table 1). Thus, it is not possible to directly determine how the choice of wiring material influences the results obtained from EPG studies with whiteflies. However, it is notable that across recent studies, the total duration of phloem sap ingestion by whiteflies tethered with gold wires rarely exceeds 60 min over a typical 6–8 h recording session, whereas studies with aphids tethered using the same wire demonstrate that phloem ingestion regularly exceeds 120 min, and often persists for over 300 min. Based on this, we hypothesized that 10–12.5 μm diameter gold wire disrupts normal whitefly probing behaviors, and that this disruption will influence the interpretation and conclusions of experiments comparing whitefly acceptance of different host plants. The present study tested this hypothesis under controlled conditions by determining whether the choice of wiring options influences the outcome of EPG experiments involving the invasive whitefly pest, *Bemisia tabaci* MEAM1 (Hemiptera: Aleyrodidae). First, we

explored the impact of the wiring choice on host-plant acceptance by comparing the probing behavior of gold wire tethered vs. platinum wire tethered *B. tabaci* on a suitable host-plant (*Vigna unguiculata*). In a second follow-up experiment, we introduced an experimental factor affecting host plant quality (application of a whitefly resistance-inducing agent, methyl jasmonate) and examined the impact of wiring choice on data interpretation and biological conclusions.

Methods

Insects and Plants

We performed all experiments using individuals from a colony of *Bemisia tabaci* MEAM1 biotype (formerly biotype B) originating from whiteflies collected in autumn 2006 from cotton at the Maricopa Agricultural Center, AZ, USA (Himler et al. 2011). *Bemisia tabaci* MEAM1 are small insects (~1 mm long) and mostly found in tropical and subtropical locations. The MEAM1 biotype is highly polyphagous and considered to be one of the most devastating insect vectors of plant viruses globally (Invasive Species Specialist Group 2018). We focused on this insect as a test case for our gold and platinum wire comparison because of its importance for agriculture, which has made it the subject of most whitefly EPG studies to date (Table 1).

We used cowpea plants (*Vigna unguiculata* cv. “CT Pinkeye Purple Hull”) as host substrates in all EPG experiments and to maintain the whitefly colony. We sowed seeds individually in starter trays, then, after successful germination, transplanted seedlings into 10*10*10 cm pots filled with UC Soil Mix 2 (Matkin and Chandler 1957). For all experiments and colony maintenance we used plants in the V3 stage (3rd trifoliolate leaf unfolded at node 5) and a 16-h light/8-h dark photoperiod under climate-controlled conditions (colony rearing at 25 ± 1 °C, EPG at 24 ± 1 °C).

To determine whether the choice of the wiring material influences the probing behavior of whiteflies on resistant and susceptible host plants (representing a typical focus of EPG studies), we treated cowpea plants with a solution of methyl jasmonate (MeJA), a known inducer of jasmonic-acid regulated defenses and whitefly resistance (Zarate et al. 2006; Walling 2008). Two days before EPG recordings, we applied MeJA solution (1.5 mM) to the adaxial leaf surface of half of the cowpea plants using hand-held atomizers (approximately 250 μ L), while the other half were treated with a control solution lacking MeJA. The MeJA solution was prepared in aqueous suspension by first making a concentrated solution of MeJA in 70% ethanol and using a small quantity of this stock diluted in water to create the 1.5 mM MeJA solution. Control solutions were prepared in the same way, but without addition of MeJA. All final solutions included 0.1% Tween 20 to ensure the solution adhered to the leaf surface instead of running off into the soil. MeJA concentration was chosen based on previous literature (Thaler et al. 1996, 2002; Traw and Bergelson 2003).

Electrical Penetration Graph Studies

We used the DC-EPG system as previously described by Tjallingii (1988) to investigate the effects of tethering material on feeding behavior of the silverleaf whitefly, *Bemisia*

tabaci on cowpea plants (*Vigna unguiculata*). To create electrical circuits that each included a plant and whitefly, we tethered each insect by attaching a thin wire (12.5 μm gold or 2.5 μm platinum) to the pronotum using conductive water-based silver glue (detailed recipe in Cervantes and Backus 2018) (Fig. 1. c and d). To facilitate the tethering process, female whiteflies were immobilized for 30–45 s at $-20\text{ }^{\circ}\text{C}$ in a freezer and placed on a Petri dish lid that was set on top of an ice pack, under a dissecting microscope. After 30 min starvation period, we positioned each whitefly on the abaxial face of the leaf (the preferred feeding location) and inserted a second electrode into the soil of each potted plant to close the electrical circuit. We recorded from eight whiteflies simultaneously over an 8-hour period of the photophase using a Giga-8 DC-EPG amplifier. Each whitefly-plant system was housed inside a Faraday cage located in a climate-controlled room held at $24 \pm 1\text{ }^{\circ}\text{C}$. We used the PROBE 3.5 software (EPG Systems, www.epgsystems.eu) to acquire and analyze EPG waveforms and relevant EPG variables (naming convention based on (Ebert et al. 2015)) were calculated with EPG-Calc 6.1 software (Giordanengo 2014). We chose variables based on five different EPG waveforms described by (Janssen et al. 1989) corresponding to: (C) stylet pathways in plant tissues except phloem and xylem; (pd) potential drops (intracellular stylet punctures); (E1) salivation in phloem elements; (E2) passive phloem sap ingestion, and (G) active xylem sap ingestion.

We carried out two EPG experiments to compare waveform information produced by tethering whiteflies with gold or platinum materials. In experiment 1, we investigated the feeding behavior of *B. tabaci* females on cowpea plants using either gold wire (12.5 μm diameter and 2 cm long) or platinum wire (2.5 μm diameter and 0.5 cm long) (Wollaston process wire; Sigmund Cohn Corp., Mt. Vernon, New York, USA). We prepared the Wollaston process platinum wire as described by Walker and Janssen (2000) and herein provide a protocol for wire preparation with diagrams (see ESM_1). For each wire type we collected 8-hour recordings from 20 individuals. In experiment 2, we designed a 2×2 factorial experiment to measure the feeding behavior of platinum-wired and gold-wired *B. tabaci* females placed on cowpea plants with two levels of resistance (resistant or susceptible). We prepared whiteflies as described above, and for each whitefly $\times$ wire $\times$ plant combination we collected 8-hour recordings from between 21 and 23 individuals.

Statistical Analyses

Mean values are given with their standard error of the mean (SEM). As whitefly feeding behavior was not normally distributed, it was analyzed using Mann-Whitney *U* tests with alpha $P < 0.05$. All statistical analyses were carried out using the statistical program “R” (version 3.2.2) (R Core Team 2016).

Results

Experiment 1: Feeding Behavior of Whiteflies Using either Gold or Platinum Wires

Platinum-wired whiteflies spent significantly more total time probing (Pr) relative to gold-wired whiteflies (Mann–Whitney *U* test; $U = 380$; $P < 0.001$) (Table 2). Platinum-

Table 2 Feeding behavior variables (all data are means per insect $\pm$ SEM) of *Bemisia tabaci* on *Vigna unguiculata* (cowpea) recorded with gold and platinum wires

EPG classes	Gold wire ($n = 20$)	Platinum wire ($n = 20$)	Statistics ^a
General probing behavior (Pr)			
1. Number of probes (#)	111.45 $\pm$ 16.02	30.8 $\pm$ 2.45	***
2. Duration of probing (min)	246.79 $\pm$ 14.99	376.61 $\pm$ 15.73	***
3. Duration of pathway phases (C) (min)	189.59 $\pm$ 14.53	200.40 $\pm$ 17.97	NS
4. Number of intracellular punctures (pd) (#)	5.55 $\pm$ 1.84	25.25 $\pm$ 4.71	***
Xylem phase (G)			
5. Number of xylem phases (#)	1.75 $\pm$ 0.46	1.70 $\pm$ 0.42	NS
*6. Duration of xylem phases (min)	64.86 $\pm$ 18.78	88.87 $\pm$ 9.83	NS
7. % of whiteflies with xylem phase	65% (13/20)	60% (12/20)	NS
Phloem phase (E)			
8. Number of phloem sap ingestion (#)	0.35 $\pm$ 0.13	1.65 $\pm$ 0.24	***
*9. Duration phloem sap ingestion (min)	31.88 $\pm$ 15.69	140.05 $\pm$ 27.59	*
10. % of whiteflies with phloem phase	30% (6/20)	85% (17/20)	NS

*: Mean $\pm$ SEM only on the individuals performing the phase

^a Asterisks and numbers in bold indicate significant differences between the two treatments (gold vs. platinum wires) (Mann–Whitney *U*-tests: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

wired whiteflies also performed significantly fewer total probes than gold-wired whiteflies (Mann–Whitney *U* test; $U = 43$; $P < 0.001$). During the pathway phase (C) platinum-wired whiteflies performed five times more intracellular punctures than gold-wired individuals (Mann–Whitney *U* test; $U = 62$; $P < 0.001$) (Fig. 2), but the total duration of pathway phases was not significantly different (Mann–Whitney *U* test; $U = 183$; $P = 0.658$). We also did not observe an effect of wiring material on the xylem phases (G), since the number of xylem phases, the mean duration per insect of xylem phases (i.e. total of all xylem phases by insect), and the number of individuals performing xylem phases were identical between treatments (Mann–Whitney *U* tests: $U = 228$; $P = 0.949$ and $U = 46$; $P = 0.087$ and Chi-squared test; $\chi^2 = 0.010$, $P = 0.922$, respectively). However, the phases in the phloem tissues (E) were strongly altered by the choice of wire. Although the percentage of whiteflies showing phloem phases was similar (Chi-squared test; $\chi^2 = 2.529$, $P = 0.112$), platinum-wired whiteflies performed almost five times more phloem-ingestion events, and fed for longer periods of time overall (per insect) (Mann–Whitney *U* tests; $U = 63.5$; $P < 0.001$ and $U = 21$; $P = 0.036$, respectively) relative to gold-wired whiteflies.

Fig. 2 Overview of an EPG by *Bemisia tabaci* on cowpea (*Vigna unguiculata*) recorded with a platinum wire. The sequence represents 10 potential drops before the beginning of a phloem phase

Experiment 2: Methyl Jasmonate Treatment and Feeding Behavior

Gold-wired whiteflies performed general probing behaviors for a shorter duration per insect and had a reduced mean duration of pathway (C) phases per insect on MeJA-treated cowpea plants in comparison to untreated control plants (Table 3a) (Mann–Whitney U test; $U = 315$; $P = 0.042$ and Mann–Whitney U test; $U = 319$; $P = 0.032$).

Table 3 Feeding behavior variables (all data are means per insect $\pm$ SEM) of *Bemisia tabaci* on *Vigna unguiculata* (treated or not with MeJA solution) recorded with a) gold and b) platinum wires

a)	Gold wire		
	CTRL ($n = 22$)	MeJA ($n = 21$)	Statistics ^a
General probing behavior (Pr)			
1. Number of probes (#)	128.45 $\pm$ 19.26	118.95 $\pm$ 10.96	NS
2. Duration of probing (min)	256.61 $\pm$ 18.06	205.12 $\pm$ 18.00	*
3. Duration of pathway phases (C) (min)	199.47 $\pm$ 12.59	158.23 $\pm$ 9.29	*
4. Number of intracellular punctures (pd) (#)	9.5 $\pm$ 3.21	5.67 $\pm$ 1.49	NS
Xylem phase (G)			
5. Number of xylem phases (#)	0.95 $\pm$ 0.21	0.90 $\pm$ 0.17	NS
*6. Duration of xylem phases (min)	61.89 $\pm$ 12.01	63.10 $\pm$ 14.00	NS
7. % of whiteflies with xylem phase	59.09% (13/22)	66.67% (14/21)	NS
Phloem phase (E)			
8. Number of phloem sap ingestion (#)	0.50 $\pm$ 0.18	0.33 $\pm$ 0.17	NS
* 9. Duration phloem sap ingestion (min)	62.18 $\pm$ 24.90	22.65 $\pm$ 6.34	NS
10. % of whiteflies with phloem phase	31.82% (7/22)	19.05% (4/21)	NS
b)	Platinum wire		
	CTRL ($n = 21$)	MeJA ($n = 23$)	Statistics ^a
General probing behavior (Pr)			
1. Number of probes (#)	65.62 $\pm$ 7.98	65.74 $\pm$ 8.32	NS
2. Duration of probing (min)	287.48 $\pm$ 21.61	301.51 $\pm$ 17.09	NS
3. Duration of pathway phases (C) (min)	166.51 $\pm$ 18.56	230.87 $\pm$ 14.42	**
4. Number of intracellular punctures (pd) (#)	15.48 $\pm$ 4.13	27.26 $\pm$ 5.76	NS
Xylem phase (G)			
5. Number of xylem phases (#)	0.71 $\pm$ 0.15	1.61 $\pm$ 0.26	**
* 6. Duration of xylem phases (min)	35.15 $\pm$ 5.15	63.59 $\pm$ 10.55	NS
7. % of whiteflies with xylem phase	57.14% (12/21)	86.96% (20/23)	NS
Phloem phase (E)			
8. Number of phloem sap ingestion (#)	1.33 $\pm$ 0.19	0.52 $\pm$ 0.24	***
* 9. Duration phloem sap ingestion (min)	109.91 $\pm$ 29.55	55.20 $\pm$ 21.18	NS
10. % of whiteflies with phloem phase	90.48% (19/21)	26.09% (6/23)	*

*: Mean $\pm$ SEM only on the individuals performing the phase

^a Asterisks and numbers in bold indicate significant differences between the two treatments (control vs. MeJA-treated plants) (Mann–Whitney U -tests: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

Xylem and phloem variables were not affected by the MeJA treatment (Mann–Whitney U tests; $P > 0.05$). In contrast, platinum-wired whiteflies exhibited changes in general probing behavior, xylem phases, and phloem phases in response to MeJA treatment (Table 3b). Platinum-wired whiteflies performed pathway phases for a full hour longer per insect on MeJA-treated plants relative to control plants (Mann–Whitney U test; $U = 131$; $P = 0.009$) and performed more xylem phases per insect on MeJA-treated plants (Mann–Whitney U test; $U = 133.5$; $P = 0.007$). Platinum-wired whiteflies also achieved two times fewer phloem ingestion events on MeJA-treated cowpeas in comparison to control plants (Mann–Whitney U test: $U = 390$; $P < 0.001$). Using platinum wire as the tether, we also observed that three times fewer whiteflies achieved phloem phases on MeJA-treated plants relative to control plants (Chi-squared test; $\chi^2 = 4.134$, $P = 0.042$). Number of intracellular punctures was not affected by the MeJA treatment for both wiring options (Mann–Whitney U tests; gold: $U = 264$; $P = 0.421$ and platinum: $U = 167.5$; $P = 0.084$).

Discussion

When considering small insects such as whiteflies, for which the EPG technique is becoming a routine tool, nearly all studies employ a variation of the “aphid standard” protocol (Cervantes and Backus 2018) using gold wires as the conductive tethering line (Table 1). An alternative 2.5 μm diameter platinum wire has been used in a subset of whitefly EPG studies (Walker and Perring 1994; Johnson and Walker 1999), but since these reports are mostly limited to waveform descriptions, there are insufficient data available to evaluate the relative tethering effects of gold vs. platinum wiring options on quantitative variables used as measures of whitefly host plant acceptance. To address this crucial methodological shortcoming, we tested the hypothesis that 12.5 μm diameter gold wire disrupts whitefly probing behaviors in comparison to 2.5 μm diameter platinum wire, and that this disruption will influence the interpretation and conclusions of experiments comparing whitefly acceptance of host plants exhibiting subtle differences in quality and palatability.

Consistent with our main hypothesis, we observed that on a suitable host plant, tethering whiteflies with gold wire reduced variables indicative of host plant acceptance by the whiteflies compared to tethering with platinum wire. Both general probing behavior and phloem phases were different depending on wire material. Gold-wired whiteflies experienced a reduced probing duration and delayed phloem phase, and consequently, a shorter duration of phloem sap ingestion, relative to platinum-wired whiteflies (Table 2). Interestingly, the xylem phase was not affected by the wiring choice, both in terms of mean duration per insect and the number of individuals performing events in this phase, even though xylem ingestion is sometimes used as an indicator of feeding difficulty (Crompton and Ode 2010) or a strategy for maintaining water balance (Spiller et al. 1990; Pompon et al. 2011). These results suggest that gold wire tethers inhibit normal *B. tabaci* probing behavior primarily by disrupting access to the phloem and reducing the ease of passive phloem ingestion. By standardizing conditions and varying only the wiring material, our study demonstrates that disruptions to phloem sap access and ingestion occur even on a suitable host that

matches that used in the rearing environment from which experimental individuals were drawn.

To test our prediction that the wiring method will change the data interpretation and biological conclusions of a standard EPG experiment (comparing suitability of two host species, cultivars, or treatments), we performed a second set of recordings that included a host quality factor - manipulation of defense status using applications of MeJA. Previous studies have shown that induction of jasmonic acid-regulated defense pathways significantly reduces the suitability of hosts for *B. tabaci* feeding and development (Zarate et al. 2006; Cui et al. 2012; Liu et al. 2017). When whiteflies are tethered with platinum wire, effects of MeJA application on host suitability variables are clear and strongly negative, in line with these expectations. Platinum-wired whiteflies exhibited altered general probing behavior (longer pathway phase) and significantly fewer individuals established contact with the phloem during the 8-hour period (Table 3b). Although not significant due to the low number of individuals actually reaching the phloem on MeJA-treated plants ($N=6$ on MeJA vs. 19 on controls), we also observed a trend for reduced durations of phloem-sap ingestion among platinum-tethered individuals feeding on MeJA-treated plants (Table 3b). These results demonstrate that tethering with platinum wire is adequate for detecting and quantifying changes in whitefly feeding behavior in response to a relatively subtle plant manipulation (defense status), which represents a typical aim of whitefly EPG in published literature (Table 1).

In contrast to the results of our platinum wire EPG recordings, when feeding behaviors are recorded with gold wire tethers, effects of MeJA treatment are nearly absent (Table 3a). According to the gold wire study, MeJA has only minor effects on host suitability through slight alterations in the mean duration of probing per insect and the mean duration of pathway phases per insect (both reduced on MeJA treatments) (Table 3a). Moreover, the direction of these effects is somewhat in opposition to what we know about how MeJA affects host quality for whiteflies (Zarate et al. 2006; Cui et al. 2012), and what these variables convey about host suitability (Lei et al. 1999). For example, longer pathway phase durations are associated with reductions in quality because the organism spends more time searching for phloem rather than ingesting phloem sap (e.g. Lei et al. 1999). When tethered with gold wire, MeJA treatment *decreased* the mean duration per insect of pathway phases, but under platinum wire tethering, MeJA treatment *increased* the mean duration per insect of pathway phases, which is consistent with effects of MeJA on host resistance to whiteflies (Table 3). The stark contrast in outcomes for gold vs. platinum wire recordings strongly supports our original hypothesis that the choice of tethering materials will influence the biological interpretation of treatment effects. Based on the gold wire recordings and the pathway durations, we could conclude that MeJA *increases* host suitability for whiteflies, while platinum wire recordings reveal the true effect: a reduction in host suitability (Zarate et al. 2006; Cui et al. 2012).

In addition to demonstrating clear differences in experimental outcomes depending on wiring material, our study also documents wire-based tethering effects on capacity to detect whitefly-specific waveform elements. EPG recordings of whiteflies and aphids are mostly similar with the notable exception of potential drops (pds) (i.e. brief intracellular punctures). For aphids potential drops are produced within seconds following initiation of probing and are abundant throughout the pathway phase (Tjallingii

1985). But prior studies with whiteflies report no, or only very few potential drops during the pathway phase (Janssen et al. 1989; Walker and Perring 1994; Lei et al. 1997). For aphids, intracellular puncture, and associated sampling of cell contents, is presumed to be the means of host selection (early punctures, before much time is spent probing) with subsequent navigation toward phloem sieve elements occurring via detection of changes in primary metabolite levels (Tjallingii and Hogen Esch 1993). For whiteflies, the first potential drops generally occur late in the pathway phase relative to aphids (20–60 min vs. 10 s) (Johnson and Walker 1999). For this reason, it has been hypothesized that whitefly intracellular punctures might not play a role in host selection, but rather serve as the mechanism of phloem sieve element location (Johnson and Walker 1999). In our study, the average time to the first potential drop for platinum-tethered individuals that eventually reached the phloem was 71.43 ± 11.00 min, which is slightly longer than what has been observed in prior studies with *B. tabaci* feeding on lima bean (18 min), broccoli (34 min), corn (53 min), and sugar beet (20 min) (Johnson and Walker 1999). Thus, potential drop numbers for platinum-tethered individuals in our study are consistent with intracellular punctures playing a role in sieve element location, but not in host selection. However, gold-tethered individuals took on average 285.50 ± 42.50 min to produce the first potential drop. Based on this, we hypothesize that use of gold wire tethers might restrict the forward movement of the stylet within the plant, thus preventing the insect from reaching sieve elements.

In addition to restrictions on the time to first intracellular puncture, we also observed that the number of potential drops per whitefly was lower when we tethered whiteflies with gold wire (mean of 5.55 potential drops, with only half of individuals producing any potential drops at all) vs. platinum wire (mean of 25 potential drops, with some individuals producing as many as 102 potential drops) (Table 2). Potential drop values above 25 are similar to the number of potential drops observed for various species of aphids (e.g. Boquel et al. 2012). Although quantitative data on whitefly potential drop numbers are lacking due to the majority of studies being performed with gold wire (during which this waveform is often not produced), Walker and Perring (1994) reported a mean of 5.45 pds for *B. argentifolii* (now *B. tabaci* MEAM1) probing on detached lima bean leaves, with as many as 16 potential drops per individual prior to first phloem contact. This study is not entirely comparable to ours due to several methodological differences. For instance, Walker and Perring counted potential drops performed prior to the first phloem ingestion event, whereas we counted potential drops performed over an 8-hour period, which included several phloem ingestion events (Tables 2 & 3). However, the larger number of potential drops produced by platinum wire-tethered individuals in our study, and clear effects of wiring choice on initiation of this waveform, suggest that the importance of intracellular punctures for whitefly-host interactions may be underestimated primarily due to lack of studies employing tethering methods that permit individuals to perform intracellular punctures.

Our overall finding that gold wire tethering inhibits detection of biologically meaningful host treatment effects, while reducing information content of whitefly EPG recordings, has implications for multiple aspects of whitefly behavior research. Whiteflies are primarily a threat because they transmit numerous phloem-limited viruses (*Begomovirus*, *Carlavirus*, *Crinivirus*, *Ipomovirus*, and *Torradovirus* genera), many of which are emerging and economically significant pathogens of food and fiber crops

(Navas-Castillo et al. 2011). Reductions in the number of phloem contacts or phloem ingestion duration will result in reduced acquisition of virions from host plants, and so achieving this has become a main goal of whitefly control programs (Castle et al. 2017). Efforts to attenuate virus spread through development of whitefly-resistant cultivars and chemical inputs with antifeedant activity rely on accurate measurements of feeding behavior disruptions, and our results indicate that gold wire tethering is likely inadequate for providing these data.

Also related to virus transmission dynamics are studies exploring putative “manipulations” of host quality by plant viruses and subsequent effects on behavioral variables associated with virion acquisition by whitefly vectors (e.g. Liu et al. 2013a, b; Lu et al. 2017; Maluta et al. 2018). To date, all whitefly EPG studies in this research area have used 12.5 μm gold wire, and it is interesting to speculate about how the biological conclusions of these studies might vary depending on the choice of tethering materials. Plant viruses often disrupt or enhance defenses mediated by core phytohormone signaling pathways, such as the JA-regulated defenses targeted in our experiments. For example, there is substantial evidence that *Tomato yellow leaf curl virus* (TYLCV, *Begomovirus: Geminiviridae*) improves resource quality for *Bemisia tabaci*, its vector, by suppressing the JA pathway (e.g. Zhang et al. 2012; Luan et al. 2013; Shi et al. 2014). TYLCV also influences whitefly behavior directly, presumably by residing and circulating within the body after acquisition (Liu et al. 2013b; Moreno-Delafuente et al. 2013). In a recent EPG study using 12.5 μm gold wire, Liu et al. (2017) showed that effects of plant JA level on probing behavior were only evident for viruliferous whiteflies. But there were little to no effects of plant JA levels on non-phloem and phloem variables of virus-free whiteflies, which is very similar to what we observed in our MeJA experiment (Table 3a). The authors suggest that activation of the JA pathway selectively attenuates manipulative effects of TYLCV on its vector, with potentially negative consequences for vector performance and virus spread. While the results certainly do support this conclusion, we expect that replication of this study using platinum wire tethering might reveal effects of plant JA level on non-viruliferous whiteflies (as seen in our study) and possibly more pronounced effects of plant JA level on TYLCV manipulation of whitefly probing behavior. Similarly, in another study exploring direct effects of TYLCV acquisition on whitefly probing on a suitable host (eggplant), viruliferous whiteflies sustained more phloem contacts relative to virus-free whiteflies (Moreno-Delafuente et al. 2013). However, only 6/18 (~33%) virus-free individuals and 11/17 (~65%) viruliferous individuals reached the phloem, meaning most individuals tethered with gold wire (~51%) were not able to obtain nutrients from what is usually a suitable host. In our study directly comparing gold and platinum wire on a different suitable host (cowpea), we observed a similar outcome: only 6/20 (30%) individuals reached the phloem when tethered with gold wire, but this number was drastically higher when platinum wire tethering was used (17/20 or 85%) (Table 2).

Overall, our results caution against extrapolating whitefly EPG results to biologically meaningful probing behavior based on recordings collected with 12.5 μm gold wire tethers. We found that the data output changes substantially depending on whether gold wire or platinum wire is used, and these differences are enough to alter the interpretation and conclusions of a typical experiment examining the influence of plant quality on probing behavior. The results of our gold wire tether recordings are also consistent with previous gold wire studies that report low numbers of individuals

successfully achieving phloem contact and ingestion (Moreno-Delafuente et al. 2013; Ning et al. 2015). By performing recordings with platinum wire under the same conditions, we demonstrate that this tethering technique alleviates probing and/or phloem ingestion difficulties that are evident, but not discussed, in studies employing gold wire tethers (Moreno-Delafuente et al. 2013; Ning et al. 2015). Based on this, we propose that future whitefly EPG studies should be performed with platinum wire if possible, and toward this purpose we provide a visually detailed protocol here as supplemental information. Beyond gaining familiarity with platinum wire tethering techniques, another significant barrier to adoption of the platinum wire tether may be the rarity of manufacturers for the material (ESM_1). The most economical supplier (Sigmund Cohn Corp.) is a US company that only accepts payment via paper check, which makes distribution to non-US countries cumbersome. We hope that the results presented here prompt other researchers, and possibly EPG system manufacturers, to explore options for making platinum wire more widely available to the whitefly research community.

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References

- Almeida RPP, Backus EA (2004) Stylet penetration behaviors of *Graphocephala atropunctata* (Signoret) (Hemiptera, Cicadellidae): EPG waveform characterization and quantification. *Ann Entomol Soc Am* 97: 838–851
- Backus EA (1994) History, development, and applications of the AC electronic monitoring system for insect feeding. In: Ellsbury MM, Backus EA, Ullman DE (eds) History, development, and application of AC electronic insect feeding monitors. Entomological Society of America, Lanham, pp 1–51
- Backus EA, Bennett WH (2009) The AC-DC correlation monitor: new EPG design with flexible input resistors to detect both R and emf components for any piercing-sucking hemipteran. *J Insect Physiol* 55: 869–884
- Backus EA, Rangasamy M, Stamm M, Mcauslane HJ, Cherry R (2013) Waveform library for chinch bugs (Hemiptera: Heteroptera: Blissidae): characterization of electrical penetration graph waveforms at multiple input impedances. *Ann Entomol Soc Am* 106:524–539
- Bonani JP, Fereres A, Garzo E, Miranda MP, Appezzato-da-Gloria B, Lopes JRS (2010) Characterization of electrical penetration graphs of the Asian citrus psyllid, *Diaphorina citri*, in sweet orange seedlings. *Entomol Exp Appl* 134:35–49
- Boquel S, Delayen C, Couty A, Giordanengo P, Ameline A (2012) Modulation of aphid vector activity by potato virus Y on in vitro potato plants. *Plant Dis* 96:82–86
- Broekgaarden C, Riviere P, Steenhuis G, del sol Cuenca M, Kos M, Vosman B (2012) Phloem-specific resistance in *Brassica oleracea* against the whitefly *Aleyrodes proletella*. *Entomol Exp Appl* 142:153–164
- Calatayud PA, Rahbé Y, Tjallingii WF, Tertuliano M, Rü B (1994) Electrically recorded feeding behaviour of cassava mealybug on host and non-host plants. *Entomol Exp Appl* 72:219–232
- Castle S, Palumbo J, Merten P, Cowden C, Prabhaker N (2017) Effects of foliar and systemic insecticides on whitefly transmission and incidence of cucurbit yellow stunting disorder virus. *Pest Manag Sci* 73:1462–1472
- Cervantes FA, Backus EA (2018) EPG waveform library for *Graphocephala atropunctata* (Hemiptera: Cicadellidae): effect of adhesive, input resistor, and voltage levels on waveform appearance and stylet probing behaviors. *J Insect Physiol* 109:21–40

- Cervantes FA, Backus EA, Godfrey L, Wallis C, Akbar W, Clark TL, Rojas MG (2017) Correlation of electropenetrography waveforms from *Lygus lineolaris* (Hemiptera: Miridae) feeding on cotton squares with chemical evidence of inducible tannins. *J Econ Entomol* 110:2068–2075
- Civolani S, Cassanelli S, Chicca M, Rison JL, Bassi A, Alvarez JM, Annan IB, Parrella G, Giorgini M, Fano EA (2014) An EPG study of the probing behavior of adult *Bemisia tabaci* biotype Q (Hemiptera: Aleyrodidae) following exposure to cyantraniliprole. *J Econ Entomol* 107:910–919
- Cornara D, Garzo E, Morente M, Moreno A, Alba-Tercedor J, Fereres A (2018) EPG combined with micro-CT and video recording reveals new insights on the feeding behavior of *Philaenus spumarius*. *PLoS One* 13:e0199154
- Crompton DS, Ode PJ (2010) Feeding behavior analysis of the soybean aphid (Hemiptera: Aphididae) on resistant soybean ‘Dowling’. *J Econ Entomol* 103:648–653
- Cui H, Sun Y, Su J, Li C, Ge F (2012) Reduction in the fitness of *Bemisia tabaci* fed on three previously infested tomato genotypes differing in the jasmonic acid pathway. *Environ Entomol* 41:1443–1453
- Ebert TA, Backus EA, Cid M, Fereres A, Rogers ME (2015) A new SAS program for behavioral analysis of electrical penetration graph data. *Comput Electron Agric* 116:80–87
- Giordanengo P (2014) EPG-Calc: a PHP-based script to calculate electrical penetration graph (EPG) parameters. *Arthropod Plant Interact* 8:163–169
- He Y, Zhao J, Zheng Y, Weng Q, Biondi A, Desneux N, Wu K (2013) Assessment of potential sublethal effects of various insecticides on key biological traits of the tobacco whitefly, *Bemisia tabaci*. *Int J Biol Sci* 9:246–255
- He W-B, Li J, Liu S-S et al (2015) Differential profiles of direct and indirect modification of vector feeding behaviour by a plant virus. *Sci Rep* 5:7682
- Helden M Van (1995) The resistance of lettuce to the aphid *Nasonovia ribisnigri*. Wageningen Agricultural University, The Netherlands
- Himler AG, Adachi-Hagimori T, Bergen JE, Kozuch A, Kelly SE, Tabashnik BE, Chiel E, Duckworth VE, Dennehy TJ, Zchori-Fein E, Hunter MS (2011) Rapid spread of a bacterial symbiont in an invasive whitefly is driven by fitness benefits and female bias. *Science* 332:254–256
- Invasive Species Specialist Group (2018) Global Invasive Species Database. http://www.iucngisd.org/gisd/100_worst.php. Accessed 7 Aug 2018
- Janssen JAM, Tjallingii WF, van Lenteren JC (1989) Electrical recording and ultrastructure of stylet penetration by the greenhouse whitefly. *Entomol Exp Appl* 52:69–81
- Jiang YX, Walker GP (2001) Pathway phase waveform characteristics correlated with length and rate of stylet advancement and partial stylet withdrawal in AC electrical penetration graphs of adult whiteflies. *Entomol Exp Appl* 101:233–246
- Jiang YX, Walker GP (2003) Electrical penetration graphs of the nymphal stage of *Bemisia argentifolii*. *Entomol Exp Appl* 109:101–111
- Jiang YX, Walker GP (2007) Identification of phloem sieve elements as the site of resistance to silverleaf whitefly in resistant alfalfa genotypes. *Entomol Exp Appl* 125:307–320
- Jiang YX, Lei H, Collar JL, Martin B, Muñoz M, Fereres A (1999) Probing and feeding behavior of two distinct biotypes of *Bemisia tabaci* (Homoptera: Aleyrodidae) on tomato plants. *J Econ Entomol* 92:357–366
- Jiang YX, Blas CDE, Barrios L et al (2000) Correlation between whitefly (Homoptera: Aleyrodidae) feeding behavior and transmission of tomato yellow leaf curl virus. *Ann Entomol Soc Am* 93:573–579
- Jiang YX, Nombela G, Muñoz M (2001) Analysis by DC – EPG of the resistance to *Bemisia tabaci* on an mi - tomato line. *Entomol Exp Appl* 99:295–302
- Jiao X, Xie W, Guo L, Liu B, Wang S, Wu Q, Zhang Y (2014) Differing effects of cabbage and pepper on B and Q putative species of *Bemisia tabaci*. *J Pest Sci* (2004) 87:629–637
- Jiao X, Xie W, Zeng Y, Wang C, Liu B, Wang S, Wu Q, Zhang Y (2018) Lack of correlation between host choice and feeding efficiency for the B and Q putative species of *Bemisia tabaci* on four pepper genotypes. *J Pest Sci* (2004) 91:133–143
- Jin S, Chen ZM, Backus EA, Sun XL, Xiao B (2012) Characterization of EPG waveforms for the tea green leafhopper, *Empoasca vitis* Gölhe (Hemiptera: Cicadellidae), on tea plants and their correlation with stylet activities. *J Insect Physiol* 58:1235–1244
- Johnson DD, Walker GP (1999) Intracellular punctures by the adult whitefly *Bemisia argentifolii* on DC and AC electronic feeding monitors. *Entomol Exp Appl* 92:257–270
- Johnson DD, Walker GP, Creamer R (2002) Stylet penetration behavior resulting in inoculation of a semipersistently transmitted closterovirus by the whitefly *Bemisia argentifolii*. *Entomol Exp Appl* 102: 115–123

- Lei H, Tjallingii WF, Van Lenteren JC, Xu RM (1996) Stylet penetration by larvae of the greenhouse whitefly on cucumber. *Entomol Exp Appl* 79:77–84
- Lei H, Tjallingii WF, Van Lenteren JC (1997) Effect of tethering during EPG recorded probing by adults of the greenhouse whitefly. *J Appl Entomol* 121:211–217
- Lei H, Tjallingii WF, Van Lenteren JC (1998) Probing and feeding characteristics of the greenhouse whitefly in association with host-plant acceptance and whitefly strains. *Entomol Exp Appl* 88:73–80
- Lei H, Van Lenteren JC, Tjallingii WF (1999) Analysis of resistance in tomato and sweet pepper against the greenhouse whitefly using electrically monitored and visually observed probing and feeding behaviour. *Entomol Exp Appl* 92:299–309
- Lei H, Van Lenteren JC, Xu RM (2001) Effects of plant tissue on the acceptance of four greenhouse vegetable host by the greenhouse whitefly: an electrical penetration graph (EPG) study. *Eur J Entomol* 98:31–36
- Liu B, Yan F, Chu D, Pan H, Jiao X, Xie W, Wu Q, Wang S, Xu B, Zhou X, Zhang Y (2012) Difference in feeding behaviors of two invasive whiteflies on host plants with different suitability: implication for competitive displacement. *Int J Biol Sci* 8:697–706
- Liu B, Preisser EL, Chu D, Pan H, Xie W, Wang S, Wu Q, Zhou X, Zhang Y (2013a) Multiple forms of vector manipulation by a plant-infecting virus: *Bemisia tabaci* and tomato yellow leaf curl virus. *J Virol* 87: 4929–4937
- Liu B, Preisser EL, Jiao X, Pan H, Xie W, Wang S, Wu Q, Zhang Y (2013b) Plant-mediated changes in the feeding behavior of an invasive whitefly. *Environ Entomol* 42:980–986
- Liu B, Preisser EL, Shi X, Wu H, Li C, Xie W, Wang S, Wu Q, Zhang Y (2017) Plant defence negates pathogen manipulation of vector behaviour. *Funct Ecol* 31:1574–1581
- Lu S, Li J, Wang X, Song D, Bai R, Shi Y, Gu Q, Kuo YW, Falk B, Yan F (2017) A semipersistent plant virus differentially manipulates feeding behaviors of different sexes and biotypes of its whitefly vector. *Viruses* 9:4
- Luan J-B, Yao D-M, Zhang T, Walling LL, Yang M, Wang YJ, Liu SS (2013) Suppression of terpenoid synthesis in plants by a virus promotes its mutualism with vectors. *Ecol Lett* 16:390–398
- Lucini T, Panizzi AR (2016) Waveform characterization of the soybean stem feeder *Edessa mediatubunda*: overcoming the challenge of wiring pentatomids for EPG. *Entomol Exp Appl* 158:118–132
- Lucini T, Panizzi AR (2018) Electropenetrography (EPG): a breakthrough tool unveiling stink bug (Pentatomidae) feeding on plants. *Neotrop Entomol* 47:6–18
- Maluta NKP, Fereres A, Lopes JRS (2017) Settling preferences of the whitefly vector *Bemisia tabaci* on infected plants varies with virus family and transmission mode. *Entomol Exp Appl* 165:138–147
- Maluta NKP, Fereres A, Lopes JRS (2018) Plant-mediated indirect effects of two viruses with different transmission modes on *Bemisia tabaci* feeding behavior and fitness. *J Pest Sci* (2004)
- Matkin OA, Chandler PA (1957) The UC-type soil mixes. In: baker KF (ed) the U.C. system for producing healthy container-grown plants through the use of clean soil, clean stock, and sanitation. University of California, division of. *Agric Sci*:68–85
- McDaniel T, Tosh CR, Gatehouse AMR et al (2016) Novel resistance mechanisms of a wild tomato against the glasshouse whitefly. *Agron Sustain Dev* 36:1–11
- McLean DL, Kinsey MG (1964) A technique for electronically recording aphid feeding and salivation. *Nature* 202:1358–1359
- McLean DL, Kinsey MG (1967) Probing behavior of the pea aphid, *Acyrtosiphon pisum*. I. Definitive correlation of electronically recorded waveforms with aphid probing activities. *Ann Entomol Soc Am* 60: 400–405
- Moreno-Delafuente A, Garzo E, Moreno A, Fereres A (2013) A plant virus manipulates the behavior of its whitefly vector to enhance its transmission efficiency and spread. *PLoS One* 8:e61543
- Navas-Castillo J, Fiallo-Olivé E, Sánchez-Campos S (2011) Emerging virus diseases transmitted by whiteflies. *Annu Rev Phytopathol* 49:219–248
- Ning W, Shi X, Liu B, et al (2015) Transmission of tomato yellow leaf curl virus by *Bemisia tabaci* as affected by whitefly sex and biotype
- Pompon J, Quiring D, Goyer C, Giordanengo P, Pelletier Y (2011) A phloem-sap feeder mixes phloem and xylem sap to regulate osmotic potential. *J Insect Physiol* 57:1317–1322
- R Core Team (2016) R: a language and environment for statistical computing. R Found Stat Comput URL <https://www.R-project.org/>.
- Rodríguez-López MJ, Garzo E, Bonani JP, Fereres A, Fernández-Muñoz R, Moriones E (2011) Whitefly resistance traits derived from the wild tomato *Solanum pimpinellifolium* affect the preference and feeding behavior of *Bemisia tabaci* and reduce the spread of tomato yellow leaf curl virus. *Phytopathology* 101: 1191–1201

- Rodríguez-López MJ, Garzo E, Bonani JP, Fernández-Muñoz R, Moriones E, Fereres A (2012) Acylsucrose-producing tomato plants forces *Bemisia tabaci* to shift its preferred settling and feeding site. *PLoS One* 7: e33064
- Seo MJ, Yang JO, Yoon C et al (2007) Differentiation in feeding behaviour of biotypes B and Q of *Bemisia tabaci* (Homoptera: Aleyrodidae) against three insecticides. *Korean J Appl Entomol* 46:401–408
- Shao-hua LU, Jing-jing LI, Ming-yang LIU et al (2015) Comparative analysis of the competitiveness between B and Q biotypes of *Bemisia tabaci* under laboratory conditions. *Sci Agric Sin* 48:1339–1347
- Shi X, Pan H, Zhang H et al (2014) *Bemisia tabaci* Q carrying tomato yellow leaf curl virus strongly suppresses host plant defenses. *Sci Rep* 4:5230
- Spiller NJ, Koenders L, Tjallingii WF (1990) Xylem ingestion by aphids - a strategy for maintaining water balance. *Entomol Exp Appl* 55:101–104
- Thaler JS, Stout MJ, Karban R, Duffey SS (1996) Exogenous jasmonates simulate insect wounding in tomato plants (*Lycopersicon esculentum*) in the laboratory and field. *J Chem Ecol* 22:1767–1781
- Thaler JS, Karban R, Ullman DE, Boege K, Bostock RM (2002) Cross-talk between jasmonate and salicylate plant defense pathways: effects on several plant parasites. *Oecologia* 131:227–235
- Tjallingii WF (1978) Electronic recording of penetration behaviour by aphids. *Entomol Exp Appl* 24:521–530
- Tjallingii WF (1985) Electrical nature of recorded signals during stylet penetration by aphids. *Entomol Exp Appl* 38:177–186
- Tjallingii WF (1986) Wire effects on aphids during electrical recording of stylet penetration. *Entomol Exp Appl* 40:89–98
- Tjallingii WF (1988) Electrical recording of stylet penetration activities. In: *Aphids: Their Biology, Natural Enemies and Control*. World Crop Pests. Elsevier, Amsterdam, pp 95–108
- Tjallingii WF, Hogen Esch T (1993) Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. *Physiol Entomol* 18:317–328
- Tosh CR, Brogan B (2014) Control of tomato whiteflies using the confusion effect of plant odours. *Agron Sustain Dev* 35:183–193
- Traw MB, Bergelson J (2003) Interactive effects of jasmonic acid, calicylic acid, and gibberellin on induction of trichomes in Arabidopsis. *Plant Physiol* 133:1367–1375
- Walker GP, Janssen JAM (2000) Electronic recording of whitefly (Homoptera: Aleyrodidae) feeding and oviposition behavior. In: Walker GP, Backus EA (eds) *Principles and Applications of Electronic Monitoring and Other Techniques in the Study of Homopteran Feeding Behavior*, XIX Intern. Thomas Say Publications in Entomology, Entomological Society of America, Lanham, pp 172–200
- Walker GP, Perring TM (1994) Feeding and oviposition behavior of whiteflies (Homoptera: Aleyrodidae) interpreted from AC electronic feeding monitor waveforms. *Ann Entomol Soc Am* 87:363–374
- Walling LL (2008) Avoiding effective defenses: strategies employed by phloem-feeding insects. *Plant Physiol* 146:859–866
- Yang JO, Kim EH, Yoon C et al (2009) Comparison of feeding behavior of B and Q biotypes of *Bemisia tabaci* (Homoptera: Aleyrodidae) against red pepper and tomato varieties. *Korean J Appl Entomol* 48: 179–188
- Yin H, Di WXY, Xue K et al (2010) Impacts of transgenic Bt cotton on the stylet penetration behaviors of *Bemisia tabaci* biotype B: evidence from laboratory experiments. *Insect Sci* 17:344–352
- Zarate SI, Kempema LA, Walling LL (2006) Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. *Plant Physiol* 143:866–875
- Zhang T, Luan JB, Qi JF et al (2012) Begomovirus-whitefly mutualism is achieved through repression of plant defenses by a virus pathogenicity factor. *Mol Ecol* 21:1294–1304
- Zhang W, Liu B, Zhang S et al (2016) Comparison of feeding behavior between two *Bemisia tabaci* strains using EPG technique. *Sci Agric Sin* 49:2544–2552
- Zhou JS, Chen AYS, Drucker M, Lopez NH, Carpenter A, Ng JCK (2017) Whitefly feeding behavior and retention of a foregut-borne crinivirus exposed to artificial diets with different pH values. *Insect Sci* 24: 1079–1092

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