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Substrate-mediated feeding and egg-laying by spotted wing drosophila: waveform recognition and quantification via electropenetrography

Raul Narciso C. Guedes[1,2] · Felix A. Cervantes[1] · Elaine A. Backus[1] · Spencer S. Walse[1]

Abstract

Substrate suitability is a key determinant of feeding and egg-laying decisions by arthropods and rigorous observation of such activities provides important management insight. Electropenetrography (EPG) was used to analyze feeding and egg-laying by the spotted wing drosophila (*Drosophila suzukii* (Matsumura)) on artificial diet and strawberry fruits. Three behavioral phases were recognized on both substrates: non-probing, feeding, and egg-laying. The non-probing phase encompassed a family of waveforms consisting of resting (coded as Z), grooming (G), and walking (W). The feeding phase encompassed waveforms representing substrate dabbing (D) and ingestion (I), while the egg-laying phase encompassed abdominal probing (P) and egg-laying (L) per se. The egg-laying phase was similar on diet and strawberry. In contrast, non-probing events were more frequent, but shorter, leading to less overall non-probing on diet compared with strawberry. Dabbing was more frequent and lasted longer overall on diet, but ingestion events lasted longer on strawberry. Therefore, although the flies fed (dabbed and ingested) for longer overall on diet, each ingestion event was longer on strawberries. Our results suggest that strawberry fruits are a more suitable and preferred food source because they led to extended periods of sustained ingestion. These findings demonstrate the first application of EPG for characterizing substrate-specific feeding and egg-laying behaviors of a key phytophagous pest, offering intriguing insight into management as well as host selection behaviors.

Key message

- Three substrate-associated phases were recognized in adults of *Drosophila suzukii*: non-probing, feeding, and egg-laying.

- Dabbing and ingestion constituted the feeding phase, while abdominal probing and egg-laying constituted the egg-laying phase.
- The egg-laying phase was quantitatively similar on diet and strawberry.
- Non-probing was more frequent, but shorter on diet, where feeding lasted longer and was more frequent.
- Dabbing was more frequent and lasted longer on diet, while ingestion events per insect lasted longer on strawberry.

✉ Raul Narciso C. Guedes
  guedes@ufv.br

[1] USDA-ARS San Joaquin Valley Agricultural Sciences Center, 9611 S. Riverbend Avenue, Parlier, CA 93648-9757, USA

[2] Departamento de Entomologia, Universidade Federal de Viçosa, Viçosa, MG 36570-900, Brazil

Introduction

Substrate suitability is paramount for arthropods in general and agricultural pest species, in particular, as these herbivores are strictly dependent on their host plants for survival, development, and/or reproduction (Jaenike 1990; Bernays

1991; Gatehouse 2002). Host/substrate-mediated activities and adaptations are associated with the arthropod's behavioral repertoire, thus establishing the underlying mechanistic links between physiology and behavior. Feeding and egg-laying, for instance, are key components of the said repertoire that largely determine substrate colonization (Fox and Czesak 2000; Simpson et al. 2015). Therefore, the detailed dynamics of both feeding and egg-laying are of interest for mass rearing and/or management purposes; however, observation of such dynamics can be challenging for small organisms. Understanding feeding dynamics in small insects has been facilitated by the development of high-resolution, electronic recording via electropenetrography (or electrical penetration graph technology; EPG) (Backus 2000; Walker 2000). EPG is an electrobehavioral technique that records voltages from ionized fluids moving between the substrate and the insect, such as the flow of food and/or saliva through the mouthpart.

Electronic monitoring of insect feeding by means of EPG was initially conceived and developed by McLean and Kinsey (1964) and has undergone over 50 years of further development. In brief, the insect is incorporated into an electrical circuit by attaching a gold wire to its dorsum and connecting it to a head stage amplifier connected to a controller, which also electrifies the substrate (Walker 2000). The contact of the insect with the substrate closes the electrical circuit, allowing the signal to pass through the insect to the EPG controller, which amplifies and digitally records the signal as a waveform representing the output voltage fluctuation over time (Backus 1994, 2016). EPG recordings have detailed feeding across a range of phytophagous hemipteroid insects, including true bugs, scales, and aphids (i.e., Hemiptera) (e.g., Cole et al. 1993; Calatayud et al. 2001; Xue et al. 2009; Rangasamy et al. 2015; Cervantes et al. 2017), and thrips (Thysanoptera) (Harrewijn et al. 1996a, b; Kindt et al. 2003). In contrast, very few egg-laying studies have been published, only with whiteflies and aphids (Walker and Perring 1994; Tosh et al. 2003; Nam and Hardie 2012). Other phytophagous insects with different mouthparts and feeding habits have been wholly neglected, so far.

The current third generation of EPG monitors, the AC–DC electropenetrograph allows recording of arthropod–substrate interactions for a broader range of species and conditions than previous EPG monitors (Backus 1994; Tjallingii 2000; Backus and Bennett 1992, 2009), providing opportunity to evaluate feeding and egg-laying activities of non-hemipteroid species (Labandeira 1997; Atallah et al. 2014; Blanke et al. 2015), such as the spotted wing drosophila (*Drosophila suzukii* (Matsumura) (Lee et al. 2011; Rota-Stabelli et al. 2013; Haye et al. 2016). The spotted wing drosophila is a pest of concern to horticultural producers across the globe (Hauser 2011; Cini et al. 2012; Deprá et al. 2014; Asplen et al. 2015). Adults are not particularly active,

with limited flight endurance and walking activity; however, both larvae and adults are attracted to undamaged ripening fruits. This is in contrast to other *Drosophila* species, which usually only attack decaying or rotten fruits (Lee et al. 2011; Rota-Stabelli et al. 2013). As a result, the insect remains in close contact with its substrate for an extended length of time, so host selection is a key determinant of life history traits in this pest species (Lihoreau et al. 2016; Plantamp et al. 2017).

The serrated ovipositor of spotted wing drosophila with enlarged bristles apparently allows the insect to pierce through the relatively hard skin of fruits so that they can lay their eggs sub-superficially in the fruit (Atallah et al. 2014; Hamby et al. 2016). This adaptation apparently allowed the colonization and damaging of intact ripening fruits, an unusual trait among *Drosophila* fruit flies (Atallah et al. 2014; Lasa et al. 2017). The analogy of more recently evolved mouthparts exhibiting structural interaction, as in the sucking and sponging mouthparts of hemipterans and flies (including fruit flies), respectively, reinforces their close interaction between substrate surface and feeding dynamics (Labandeira 1997; Blanke et al. 2015). Such facts suggest that spotted wing drosophila would be amenable to EPG feeding studies, similar to hemipteroid insects, with the added benefit of also allowing detailed studies of the egg-laying dynamics in a species that remains in contact with its substrate for extended periods of time without flight.

Detailed study of the interaction between spotted wing drosophila and its feeding and egg-laying substrate will aid in understanding the process of host selection and acceptance, and its potential management via surface agents such as insecticides. Therefore, we aimed here to electronically monitor the interaction between adults of the spotted wing drosophila and two substrates, i.e., artificial diet and strawberry fruits. The objectives were (1) to assess the suitability of EPG for monitoring substrate-mediated activities of spotted wing drosophila; (2) to develop methods for recognizing waveforms descriptive of the main behaviors observed, and (3) to determine the qualitative and quantitative waveform differences from both substrates. We expected to be able to record and recognize the relevant waveforms associated with the exhibited behavioral activities. We also expected differences between both substrates, with the natural (preferred) host fruit favoring feeding and possibly egg-laying.

Materials and methods

Insects and substrates

Spotted wing drosophila adults were obtained from a laboratory colony at USDA-ARS Parlier, reared in nylon mesh enclosures (Bug Dorm-2®, BioQuip, Rancho Dominguez,

CA, USA) and maintained at 24–27 °C temperature, 70% relative humidity, and 16:8 hs (L/D) photoperiod. The insects were reared as previously described using corn-meal–sucrose–agar–yeast diet provided in Petri dishes for egg-laying and larvae development (Walse et al. 2012; Bellamy et al. 2013). Each nylon mesh enclosure contained about 2000 adult flies.

The substrates used in the EPG experiments were the rearing diet, provided as small slabs of diet (1 cm wide × 3 cm long × 0.5 cm thick), and strawberry fruits. The diet was prepared as previously described, and the strawberries were obtained from a local organic market. Prior to use, the fruits were washed in distilled water and air-dried before providing to the flies. Damaged and mold-infected fruits were not used in the bioassays.

Insect wiring

Gravid female adults (4- to 7-days old), selected at random from the rearing cages, were used in the EPG studies. Specimens were starved for 1 h and then chilled (10 min at 10 °C) after which, a gold wire of 25.4 µm diameter (sold as 0.001 in.; Sigmund Cohn, Mt. Vernon, NY, USA) was glued to the insect pronotum using water-based silver glue (1:1:1 [vol:vol:wt] of white glue, water, and silver flake, Inframat Advanced Materials, Manchester, CT, USA; further details in Cervantes et al. (2018)). Wiring was conducted using a stereomicroscope (MZ12₅, Leica, Heerbrugg, Switzerland), with the tip of the wire opposite the insect attached to a brass escutcheon pin for connection to the head stage amplifier.

EPG monitoring

A four-channel version of the third generation AC–DC correlation monitor (Backus and Bennett 2009) (EPG Technologies, Gainesville, FL, USA) was used for all recordings. Insect/substrate preparations and head stage amplifiers were enclosed in a Faraday cage to minimize electrical noise. This monitor operates with either alternating current (AC) or direct current (DC) and tunable input impedance (amplifier sensitivity) (from 10^6 to 10^{13} Ω), affording an opportunity for operational optimization across diverse species and recording conditions. Each wired insect was connected to an individual head stage amplifier and placed either on a small diet slab or a strawberry, with insects on each substrate simultaneously recorded side-by-side. Each substrate had a copper electrode inserted (1.0 cm long), which was connected to the EPG monitor closing the circuit.

The changes in electrical current were amplified, rectified, and digitized at a sample rate of 100 Hz per channel using a DI-720 analog-to-digital board (Dataq Instruments, Akron, OH, USA), and recorded on a desktop computer equipped with WinDaq Pro+ software (Dataq). The EPG settings were established after preliminary tests of multiple input resistor (Ri) or impedance and applied signal levels, leading to our choice of 10^9 Ω and a standardized voltage of 20 mV AC. DC was also tested; it provided optimum signal-to-noise ratios under the same conditions as with AC, but the waveforms were not as consistent and detailed in appearance. A recording period of 4 h was used, starting at mid-morning (10:00 am).

Both pre- and post-rectification output signals were simultaneously recorded and checked for proper use of the offset function of the monitor, to avoid rectifier fold-over of the output signal. However, only the post-rectification signal was measured for all the recordings. The monitor gain for the recording was 3000×, and the WinDaq gain ranged from 8× to 16×. The female insect interactions with the substrate recorded with the EPG monitor were also observed with a digital camera (DFC7000 T) coupled with a MZ125 stereomicroscope and a desktop computer equipped with the LAS X image recording software (all from Leica, Heerbrugg, Switzerland), to correlate visual observations of behavioral activities with corresponding waveforms.

Behavior (and waveform) quantification

A total of 26 and 30 female insects were observed and recorded interacting with diet and strawberry, respectively. The waveforms representing the recorded behaviors were named following the terminological conventions earlier proposed (Backus 1994; Backus et al. 2007), adhering to a hierarchical scheme from phase to family. The waveform categories were quantified based on stereotypical patterns where a continuously uninterrupted waveform occurrence was termed a waveform event. The number of events and their durations were measured using WinDaq Waveform Browser software (also from Dataq Instruments) (Serrano et al. 2000). Three main, non-sequential response variables were calculated, following the naming convention of Backus et al. (2007). The sum of all events of a given waveform was averaged per insect to provide the waveform duration per insect (WDI). The mean number of waveform events per insect (NWEI) and mean duration of waveform events per insect (WDEI) for each waveform (phase and family) were also determined.

Statistical analyses

Descriptive statistics were calculated using the Backus 2.0 program (available at http://www.crec.ifas.ufl.edu/extension/epg/epg_workshop.shtml) developed for Statistical Analysis Software (SAS, Cary, NC, USA) (Backus et al. 2007; Ebert et al. 2015). Analysis of variance using restricted maximum likelihood estimation (REML-ANOVA) was performed using the procedure GLIMMIX for SAS, to determine

whether differences of measured variables observed for diet and strawberry substrates (fixed variable) were significant; no random variable was specified. REML-ANOVA was used because of its flexible use compared with conventional ANOVA, particularly regarding no assumption of normality, as well as its higher power compared with alternative tests (e.g., Mann–Whitney *U* test) (Ebert et al. 2015). All analyses were performed at the phase level and also at family-level when desired. No data transformation was necessary to improve homoscedasticity.

Results

Summary of behaviors and waveform phases

The substrate-associated behaviors of adult females of spotted wing drosophila were viewed using stereomicroscopy and visually correlated with three distinct phases based on their waveform coarse structure. The phases included a non-probing phase, a feeding phase, and an egg-laying phase, each with a corresponding set of waveform families associated with specific behavioral patterns and the general characteristics summarized in Table 1. The general appearances of the three waveform phases are representatively depicted in Fig. 1.

The non-probing phase encompassed three sets of behaviors and respective waveforms (coded as capital letters in parenthesis): resting (coded as Z), grooming (G), and walking (W). In contrast, the feeding and egg-laying phases each consisted of two behaviors and corresponding

waveforms. Dabbing (D) and ingestion (I) were the behaviors/waveforms during the feeding phase, while abdominal probing (P) and egg-laying per se (L) were the behaviors/waveforms constituting the egg-laying phase. An additional waveform was also recognized as a brief interruption taking place during the events of feeding and/or egg-laying phases, not lasting more than 1.5 s, and was coded as N.

Waveform characterization

Non-probing phase

Waveforms of the non-probing phase exhibited relatively lower amplitudes and irregular frequencies compared with those of the other phases, particularly the egg-laying phase (Table 1, Fig. 2). Resting and the corresponding Z waveform were observed when the fly was standing/resting on the substrate surface. Regardless of the substrate, whether diet or strawberry, Z was characterized by very low amplitude or, most frequently, a flat line without visible changes. Thus, Z is also known as the baseline of each recording (Figs. 1, 2).

Grooming corresponded to a diverse set of (primarily) leg movements always aimed at cleaning or removing foreign material from body surfaces. Leg activities may have taken place on the head, abdomen, legs themselves, or wings, eliciting differences in irregular waveforms. However, these G waveforms were of low amplitude and rounded, not as peaked nor as tall as walking, which also was characterized

Table 1 Summary of each EPG waveform representative of non-probing and probing behaviors of adult female spotted wing drosophila (*Drosophila suzukii*) ($n = 10$)

Phase	Family (coding)	Relative amplitude (%)		Frequency (Hz)		Biological meaning
		Diet	Strawberry	Diet	Strawberry	
Non-probing	Resting (Z)	Flat	Flat	Flat	Flat	No movement or activity
	Grooming (G)	14.0 ± 2.3	18.7 ± 3.1	Irregular	Irregular	Grooming of body parts (either head, abdomen, legs, or wings)
	Walking (W)	38.1 ± 2.1	42.8 ± 3.0	Irregular	Irregular	Walking on substrate surface
Feeding	Dabbing (D)	41.3 ± 4.1	44.6 ± 3.6	0.9 ± 0.1	0.9 ± 0.1	Brief substrate touch by the spongiform labellum with proboscis extension
	Ingestion (I)	41.2 ± 3.2	40.6 ± 2.7	Irregular	Irregular	Extended substrate touch by labellum with proboscis extension
Egg-laying	Probing (P)	90.4 ± 3.5	90.9 ± 2.7	0.9 ± 0.1	0.9 ± 0.1	Brief touch and insertion of ovipositor into the substrate
	Egg-laying (L)	100.0 ± 4.5	100.0 ± 3.5	Irregular	Irregular	Sustained insertion of ovipositor into substrate with the laying of an egg
Other	Interruption (N)	Very low	Very low			Brief interruption during the behaviors of the feeding or egg-laying phases

The AC–DC EPG settings were 20 mV AC (alternate current) applied voltage and 10^9 Ω input impedance. The mean values ($\pm$ SE) of frequency and amplitude are from 10 events for each of at least four insects. Amplitude values were standardized by the highest amplitude value (i.e., that of egg-laying) for estimation of the relative amplitude (%)
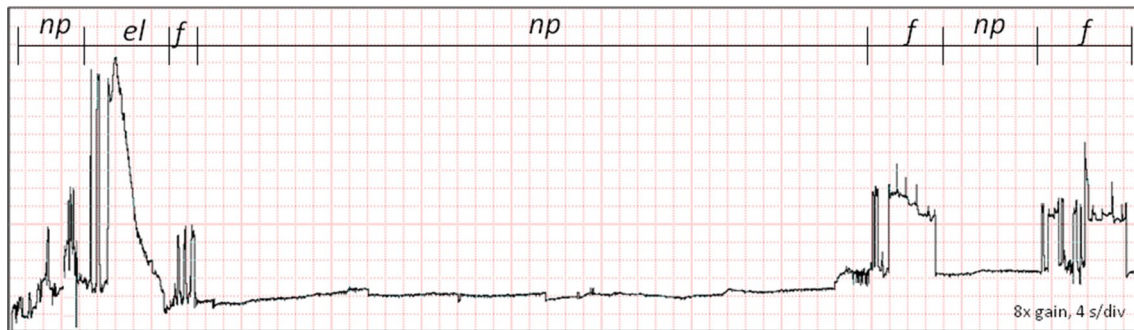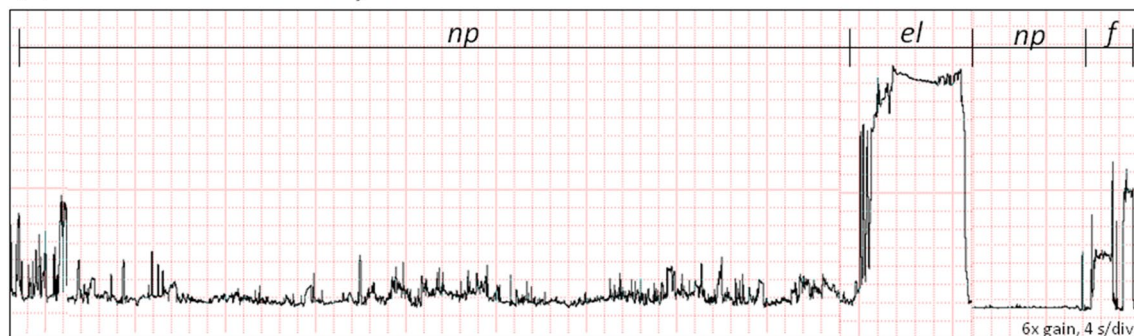
Fig. 1 Overview of representative EPG waveforms produced for adult female of spotted wing drosophila (*Drosophila suzukii*) with 20 mV AC applied signal on diet (**a**) and strawberry (**b**) using 10^9 Ω (Ri). Monitor gain was set at 3000×, and WinDaq gains are indicated in each recording, as are the *x*-axis compressions. The waveform phases are indicated and coded as non-probing (*np*), feeding (*f*), and egg-laying (*el*)

by irregular waveforms. Walking waveforms (W) were subject to acute peaks and valleys of high amplitude that frequently reached those of waveforms in the feeding phase, but not in the egg-laying phase (Fig. 2).

Feeding phase

Dabbing (D) and ingestion (I) were characterized by distinct stereotypical waveforms of similar amplitude, regardless of the substrate (Fig. 3). The main difference was in the duration per event, as dabbing seldom lasted more than a second (< 1.5 s), unlike feeding, which was a sustained activity and thus each event lasted longer (i.e., < 1.5 s, usually > 3 s), even when relatively brief. D consisted of a peak with a relatively flat plateau containing some irregularities. In contrast, I consisted of blocks formed by sudden voltage changes and relatively flat plateaus. Nonetheless, the relatively flat-peaked plateaus of ingestion frequently exhibited periodic low-amplitude drops or spikes of voltage that greatly varied among individual flies and substrates (Fig. 3). Video/visual observations revealed that the plateau portion of D and I corresponded to the contact maintained by the extended insect proboscis with the feeding substrate.

Egg-laying phase

This phase constituted abdominal probing (P) and egg-laying (L). Both waveforms exhibited similar amplitude, although L was usually 10% higher and lasted longer (> 15 s), representing ovipositor insertion into the substrate with partial to complete insertion of an egg into the substrate (Table 1, Fig. 4). In contrast, P was just a brief touch and ovipositor insertion not lasting more than one second and without laying any egg (Fig. 4). P frequently preceded L, but both also occur independently of each other. Unlike P, the appearance of waveform L differed between substrates, exhibiting a slow, steady decline on diet but a flat plateau followed by a sharp decline on strawberry (Fig. 4).

Quantitative substrate-mediated differences

Waveform phases

Numbers and durations of non-probing and feeding events were significantly different on diet versus strawberry substrate (REML-ANOVA: $F_{1,53} > 4.03$, $P < 0.05$), but no differences were observed during egg-laying phase
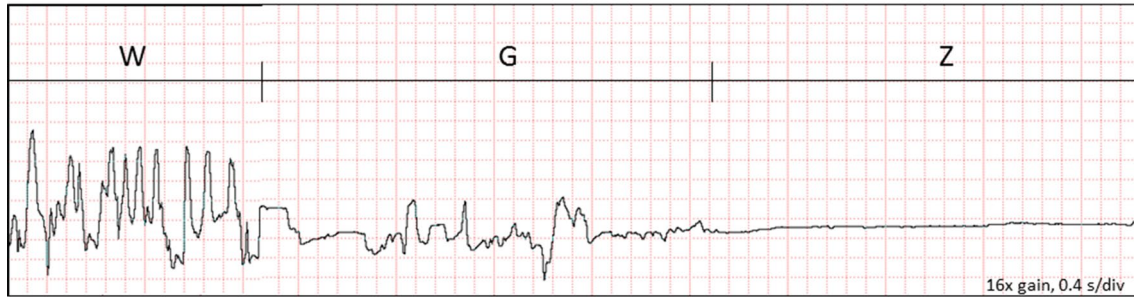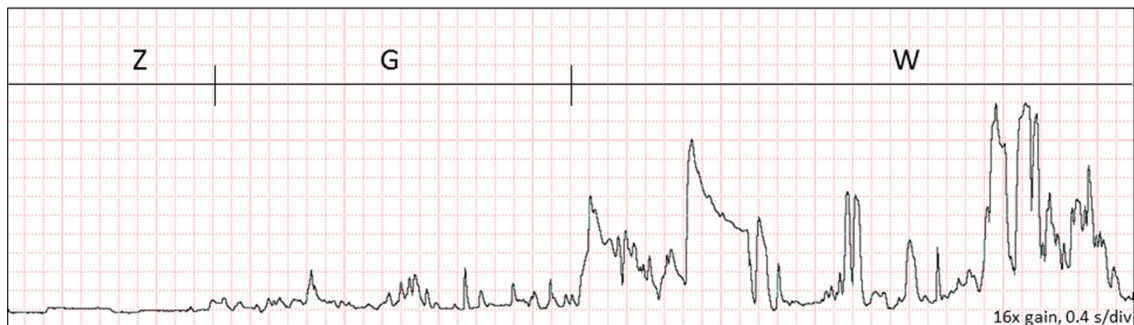
(A) Non-probing on diet

(B) Non-probing on strawberry

Fig. 2 Overview of representative EPG waveforms produced during the non-probing phase of adult female spotted wing drosophila (*Drosophila suzukii*) with 20 mV AC applied signal on diet (**a**) and strawberry (**b**) using 10^9 Ω (Ri). Monitor gain was set at 3000×, and WinDaq gains are indicated in each recording, as are the *x*-axis compressions. The waveforms from the non-probing phase are indicated and coded as resting (Z), grooming (G), and walking (W)

(REML-ANOVA: $F_{1,28} \leq 1.57$, $P \geq 0.82$). The pooled mean ± SE for abdominal probing and egg-laying were, respectively: 8.70 ± 2.49 and 3.18 ± 1.09 events per insect (NWEI); 17.43 ± 9.15 and 78.96 ± 33.48 s of overall duration per insect (WDI); and 0.66 ± 0.22 and 13.90 ± 2.17 s of event duration per insect (WDEI). Regarding the non-probing phase, the number of waveform events per insect (NWEI) was greater on diet (Fig. 5a), while the opposite occurred for the overall duration of non-probing per insect (WDI) (Fig. 5b) and the waveform duration of each non-probing event per insect (WDEI) (Fig. 5c). The trend was different for feeding phase, with the number of events and overall phase duration per insect higher on diet than on strawberry (Fig. 6a, b), but without significant differences in waveform duration per feeding event per insect (pooled mean ± SE = 8.90 ± 2.10; REML-ANOVA: $F_{1,53} = 1.46$, $P = 0.23$). Therefore, strawberry led to longer resting events/intervals accounting for more resting overall, but in lower numbers of events. In contrast, the number of feeding events and the time spent feeding (overall) were higher on diet.

Waveform families

The patterns of dabbing and ingestion differed between substrates (REML-ANOVA: $F_{1,53} > 7.94$, $P < 0.05$). The number of dabbing events per insect was greater, and the overall length of time the insects spent dabbing was longer when on diet, as compared with strawberry (Fig. 7). However, event duration per insect for dabbing did not differ between substrates (REML-ANOVA: $F_{1,53} = 0.10$, $P = 0.75$); thus, the increased overall duration was caused by a greater number of dabbing events. Ingestion also differed between diet and strawberry, with greater number of ingestion events (Fig. 8a) and overall duration of ingesting (Fig. 8b) on diet rather than on strawberry. In contrast, the duration of each ingestion event was longer for insects on strawberry (Fig. 8c). Thus, strawberry supported fewer, longer ingestion events, while diet led to more frequent ingestion events of shorter durations, summing to a longer overall duration spent ingesting on diet.
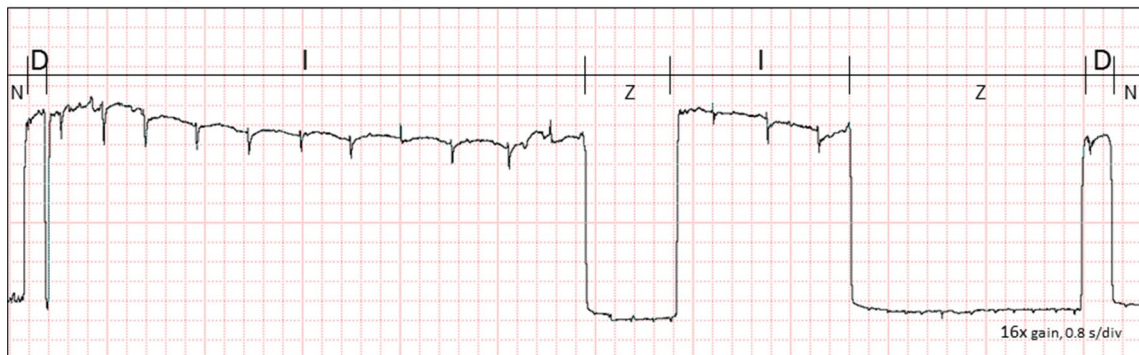
(A) Feeding on diet

(B) Feeding on strawberry

Fig. 3 Overview of representative EPG waveforms produced during the feeding phase of adult female spotted wing drosophila (*Drosophila suzukii*) with 20 mV AC applied signal on diet (**a**) and strawberry (**b**) using 10^9 Ω (Ri). Monitor gain was set at 3000×, and WinDaq gains are indicated in each recording, as are the *x*-axis compressions. The waveforms from the feeding phase are indicated (and coded) as dabbing (D), and ingesting (I); waveforms from other phases are also indicated, but below the reference line and with smaller font size (N for interruption, G for grooming, and Z for resting)

Discussion

The feeding and egg-laying behavior of adult female spotted wing drosophila on different substrates, diet and strawberry, was evaluated with EPG. We expected waveform differences between substrates due to intrinsic differences in consistency, appearance, sugar and nutrient contents, and also semiochemicals present. Indeed, our expectations were largely confirmed.

Waveform characteristics and biological meaning

EPG recordings showed seven characteristically distinct waveforms associated with three activity phases—non-probing, feeding, and egg-laying—in addition to an eighth waveform representing the interruption of feeding and/or egg-laying waveforms (interruption, N; or brief return to baseline between waveforms). These eight waveforms were detected with an applied voltage of 20 mV and visible at different impedances (Ri), although at lower amplitude Ri decreased below 10^9 Ω. This observation suggests that the electrical origins of all waveforms are mainly due to fluctuations of electromotive forces (emf) (i.e., voltage oscillations

or biopotentials generated by biological processes of the arthropod or its substrate) (Walker 2000). These detected waveforms were consistent with those of other species (e.g., Cervantes et al. 2017). Waveforms from the feeding and egg-laying phases exhibited consistent shape and magnitude, while the non-probing waveforms of grooming (G) and walking (W) were irregular and variable.

The non-probing phase was characterized by three activities and respective waveforms: resting (Z), walking (W), and grooming (G). Waveform Z was nearly flat, showing almost no voltage oscillation and therefore consistent with the observed resting behavior of the insects where no activity is apparent (similar to resting waveforms in Backus 2000; Youn et al. 2011; Rangasamy et al. 2015; Cervantes et al. 2017; Lucini and Panizzi 2017). Walking, recorded as waveform W, was highly variable with acute peaks and valleys at amplitude ranges reaching and even surpassing those of the feeding phase, as also observed for true bugs and the Asian citrus psyllid, *Diaphorina citri* (Backus et al. 2007; Youn et al. 2011). The structural features of the adult fly tarsi and its close contact with the substrate while scratching, grasping or merely touching it, likely generate small electrical currents between the insect and the electrified

Fig. 4 Overview of representative EPG waveforms produced during the egg-laying phase of adult female spotted wing drosophila (*Drosophila suzukii*) with 20 mV AC applied signal on diet (**a**) and strawberry (**b**) using 10^9 Ω (Ri). Monitor gain was set at 3000×, and WinDaq gains are indicated in each recording, as are the *x*-axis compressions. The waveforms from the egg-laying phase are indicated and coded as probing (P), and egg-laying per se (L); waveforms from other phases are also indicated, but below the reference line and with smaller font size (N for interruption, G for grooming, and Z for resting)

(A) Egg-laying on diet

16x gain, 0.4 s/div

(B) Egg-laying on strawberry

8x gain, 0.4 s/div
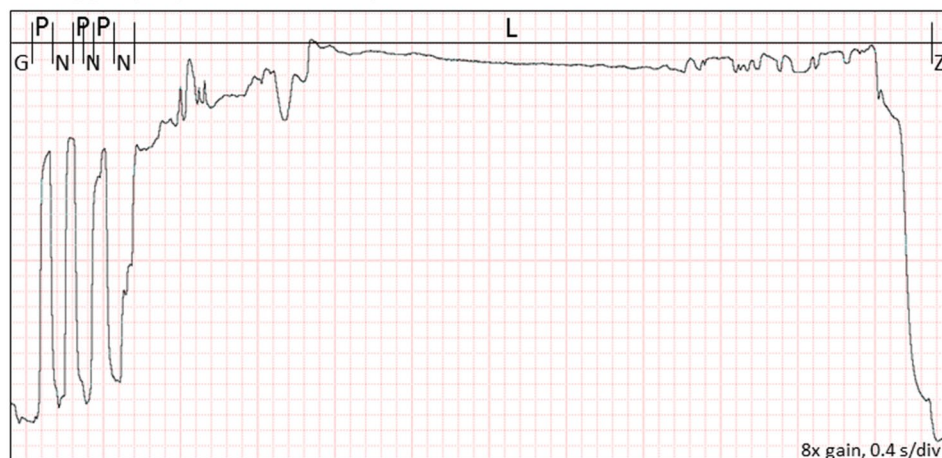
substrate leading to the waveform W. A similar rationale is valid for grooming and its waveform G. However, in this case, variability was even higher and the range of amplitude was usually lower with more rounded peaks, although higher amplitudes may take place and even reach those associated with the feeding waveforms. Because the flies performed a variety of grooming activities for potentially long durations, waveform G may likely be further discriminated/characterized in subtypes in a future study.

Feeding, partitioned into either dabbing or ingestion, differed in duration, not in amplitude, between the substrates. Waveform D, formed from contact between substrate and the (fly) proboscis labellum, was characterized by brief (< 1.5 s) rectangular peaks, while waveform I was inversely rectangular (i.e., lower height and longer length) and lasted relatively longer, usually over 5 s (mean of 11.45 ± 2.3 s on diet and over twice that on strawberry). In contrast, the dynamics of ingestion by the fly likely accounted for the longer duration and regular plateau features (Itskov et al. 2014), minor valleys and/or spikes, on the top of waveform I. Similar recordings were also obtained with the proboscis activity detector of Itskov et al. (2014); however, waveform

events of dabbing and ingesting during the feeding phase were not distinguished.

Fruit flies use spongiform mouthparts and flexing of the proboscis to feed, during which a fluid-centered mechanism with regurgitation and re-ingestion takes place (Vijaysegaran et al. 1997). Fruit flies regurgitate by releasing fluid containing water, salivary enzymes, and/or symbiotic bacteria from their crop onto the surface of the feeding substrate (Vijaysegaran et al. 1997; Coronado-Gonzalez et al. 2008; Stoffolano and Haselton 2013) by means of the crop lobes pump (Stoffolano and Haselton 2013). Re-ingesting the fluid with added nutrients from the substrate surface seems to be achieved through the crop cibarial pump (Stoffolano and Haselton 2013). Regardless, the pressure variation caused by the fluid pumping from the crop pumps likely caused the voltage variation present on waveform I.

Egg-laying was characterized by abdominal probing, recorded as waveform P with a brief (< 1 s) high amplitude spike and a rounded peak, in short sequences and sometimes preceding egg-laying per se. In contrast, the egg-laying waveform, L, was of about the same amplitude as P, but more complex and lasting longer (> 10 s). Waveform
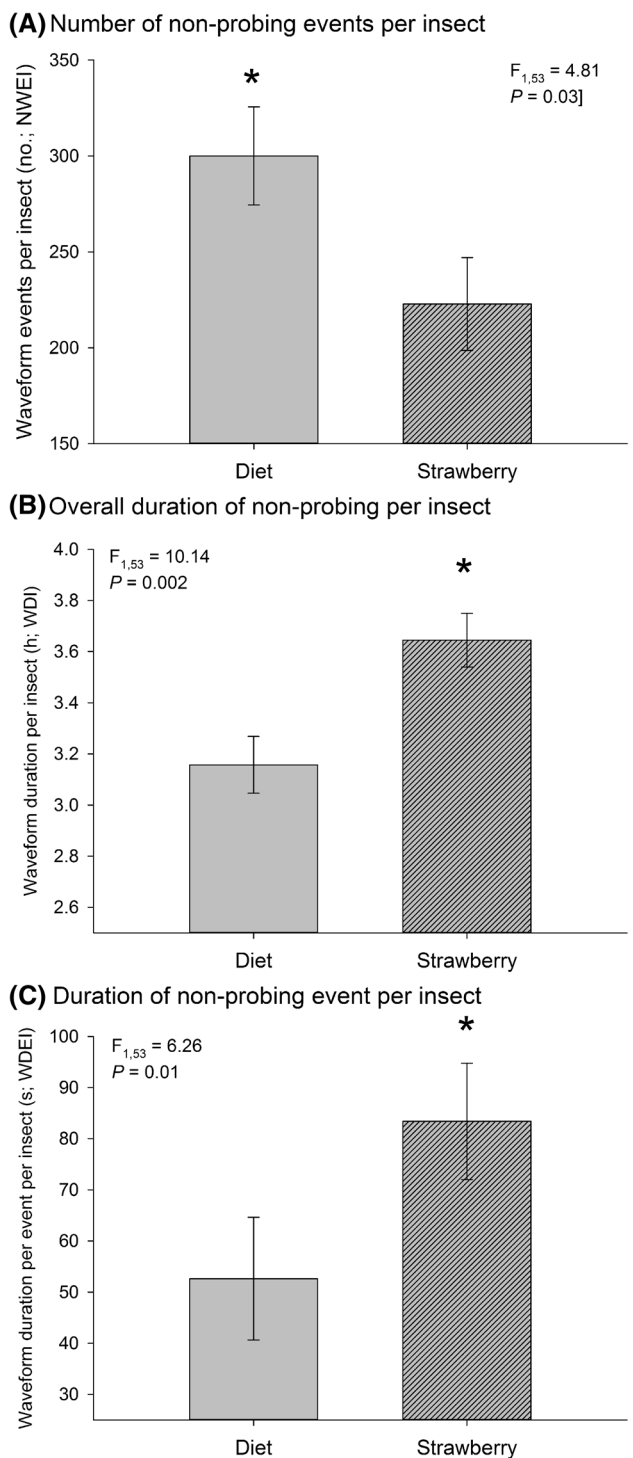
(A) Number of non-probing events per insect

(B) Overall duration of non-probing per insect

(C) Duration of non-probing event per insect

Fig. 5 Bar plots of frequency (**a**) and duration (mean ± SE) (**b**, **c**) of waveforms associated with the non-probing phase of adult female spotted wing drosophila (*Drosophila suzukii*) on diet and strawberry. The asterisk indicates significant difference by Fisher's F test (REML-ANOVA; $P < 0.05$)
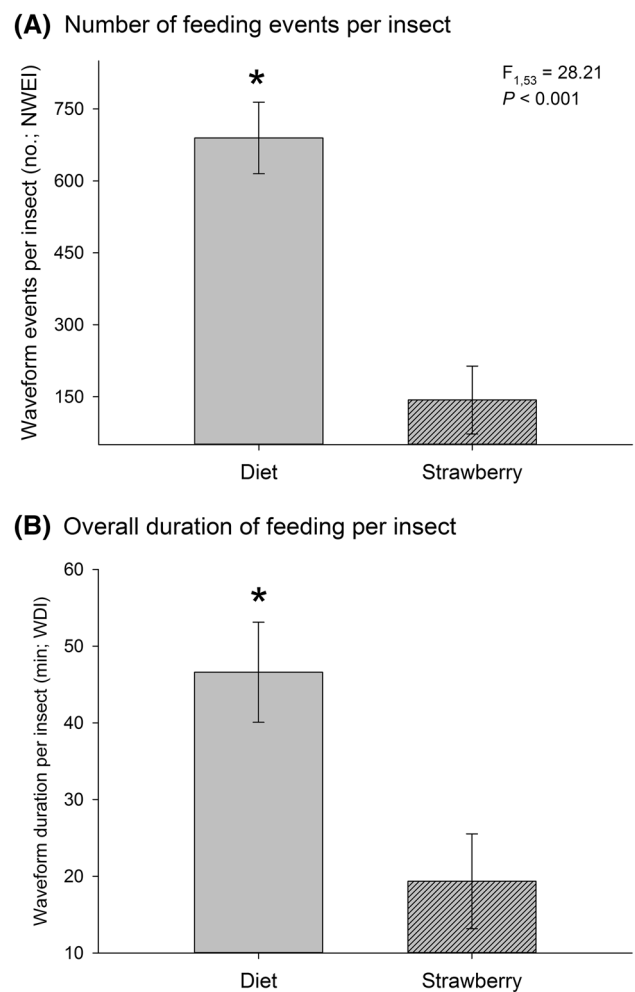
(A) Number of feeding events per insect

(B) Overall duration of feeding per insect

Fig. 6 Bar plots of frequency (**a**) and duration (mean ± SE) (**b**, **c**) of waveforms associated with the feeding phase of adult females spotted wing drosophila (*Drosophila suzukii*) on diet and strawberry. The asterisk indicates significant difference by Fisher's F test (REML-ANOVA; $P < 0.05$)

L also differed in the shape of its decline according to the substrate—abrupt decline from a plateau in strawberry and steady decline on diet. An association between waveform L and the fly ovipositor and egg-laying activity was to be expected. The serrated ovipositor of the spotted wing drosophila is one of its peculiarities allegedly allowing the surface piercing of (undamaged) live and ripe fruits (Lee et al. 2011; Rota-Stabelli et al. 2013; Lasa et al. 2017).

The spotted wing drosophila is indeed able to pierce undamaged skin of cherry and berries and even the tougher skin of grapes, in contrast with most of the other species of the genus (Atallah et al. 2014). Nonetheless, the serrated ovipositor with enlarged thorn bristles would suggest a greater effort in inserting the ovipositor into the egg-laying substrate, probably generating a graded oscillation
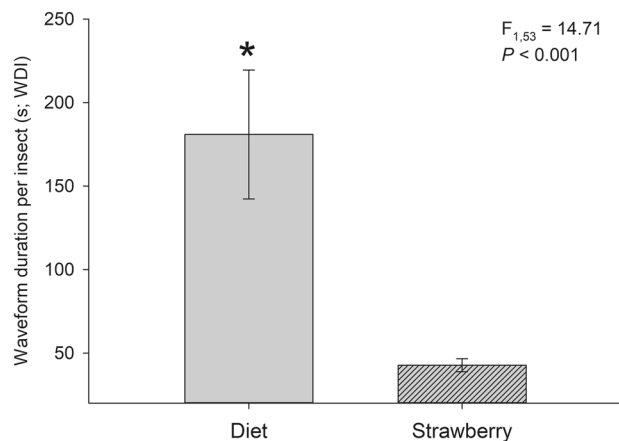
(A) Number of dabbing events per insect

$F_{1,53} = 17.49$
$P < 0.001$

(B) Overal duration of dabbing per insect

$F_{1,53} = 14.71$
$P < 0.001$

Fig. 7 Bar plots of frequency (**a**) and duration (mean ± SE) (**b**) of dabbing (waveform D) associated with the feeding phase of adult female spotted wing drosophila (*Drosophila suzukii*) on diet and strawberry. The asterisk indicates significant difference by Fisher's *F* test (REML-ANOVA; *P* < 0.05)

(A) Number of ingestion events per insect

$F_{1,53} = 36.73$
$P < 0.001$

(B) Overall duration of Ingestion per insect

$F_{1,53} = 7.94$
$P = 0.007$

(C) Duration of ingestion event per insect

$F_{1,53} = 6.54$
$P = 0.01$

Fig. 8 Bar plots of frequency (**a**) and duration (mean ± SE) (**b**, **c**) of ingestion (waveform I) associated with the feeding phase of adult females spotted wing drosophila (*Drosophila suzukii*) on diet and strawberry. The asterisk indicates significant difference by Fisher's *F* test (REML-ANOVA; *P* < 0.05)

in pressure (and voltage) until eventual oviposition. Curiously, waveform L from diet and strawberry did not match this expectation and indicated a rather rapid insertion of the ovipositor corresponding to a steep spike. Stewart et al. (2014) also provided evidence of rapid egg-laying by spotted wing drosophila, although suggesting the use of the fly's serrated ovipositor to break the fruit exocarp; this is also a distinction from the expected sawing movements allegedly associated with egg-laying by the spotted wing drosophila. Thus, insertion of just a terminal thorn bristle is likely enough to allow electrical conductance and waveform onset without multiple serrations, suggesting the need for a better understanding of the egg-laying dynamics involved both on preferred and non-preferred hosts. The fact that spotted wing drosophila lays eggs on
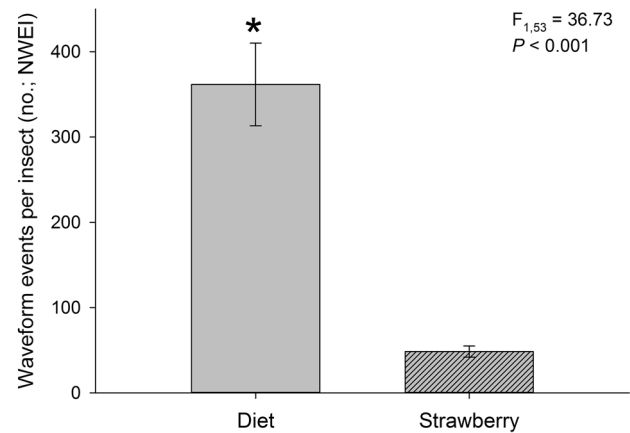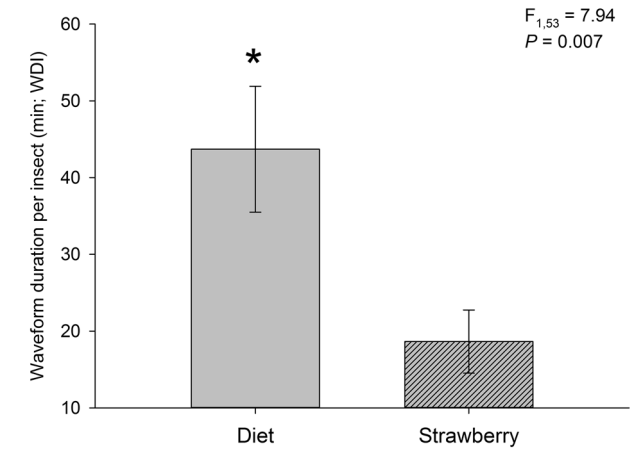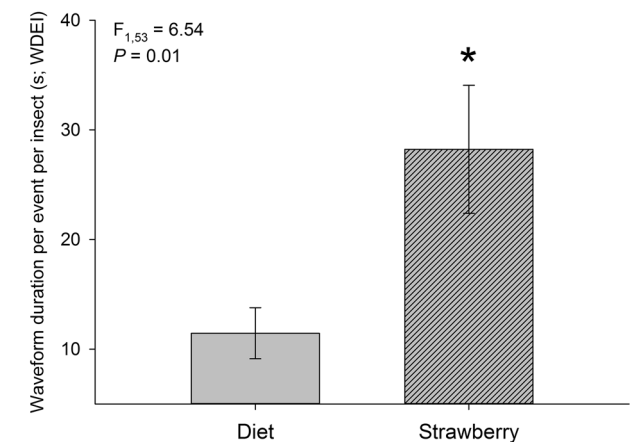
the substrate sub-surface or surface (not covering the white threads projected from one end of the egg) also challenges the notion of a sawing ovipositor insertion, although it may be important for host with thicker skin (i.e., exocarp). The declining-voltage portion of waveform L on diet may, however, represent a more difficult (serrated) ovipositor retraction from the substrate, unlike in strawberry.

Quantitative substrate-mediated differences

Egg-laying activity was similar in both substrates, although waveform L differed in shape between them. However, the lack of substrate differences in abdominal probing and egg-laying was probably due to the low occurrence of such activities within the 4-h recording period used in our study. Longer recording periods extended to the circadian rhythm will likely allow eventual detection of substrate-mediated egg-laying preferences in spotted wing drosophila, which may differ based on feeding preferences of the species (Lihoreau et al. 2016; Plantamp et al. 2017). In contrast, in our study, substrate led to significant differences in non-probing behavior and feeding.

Non-probing behavior lasted longer on strawberry, albeit for a smaller number of events, than on diet. Thus, feeding activity differed between these two substrates with the flies remaining in feeding for longer and with a larger number of feeding events on diet than on strawberry. Nonetheless, the duration of ingestion events lasted significantly longer on strawberry, reaching an average of nearly 3× higher than on diet. Therefore, although the flies fed for longer overall on diet, both dabbing and ingesting in short events, our results suggest that strawberry fruits are a more suitable and preferred food source leading to longer events of sustained ingestion with extended inter-feeding intervals. Such a feeding pattern, supported by waveform recordings, will allow future, high-resolution investigation of host suitability and preferences, which are important components to aid the managing this invasive pest species (Bellamy et al. 2013; Hamby et al. 2016; Haye et al. 2016).

The new reality of electronic recording of substrate-mediated feeding and egg-laying differences in spotted wing drosophila has the potential to provided additional information relevant to the management of this species. Besides tracking host preferences, feeding and egg-laying substrates are amenable to modifications. For instance, the use of fertilizers or even changes in irrigation regime may alter the substrate surface, thereby enhancing or compromising acceptance. This topic deserves future research attention. Pesticide residues on the substrate also could lead to a range of consequences for the adult flies beyond mortality, because sublethal exposure is usually as important and frequently more important than lethal exposure (Guedes et al. 2016, 2017, 2018). Therefore, the EPG approach described herein will

be a valuable potential tool for exploring and understanding such possible effects.

Author contribution

RNCG, FAC, EAB, and SSW conceived and designed the study. RNCG, FAC, and EAB established the experimental protocols, and EAB and SSW provided reagents and analytical tools. RNCG performed the experiments and analyzed the data. RNCG, FAC and EAB structured the manuscript, and RNCG wrote the manuscript. All of the authors read, corrected, and approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and institutional guidelines for the care and use of animals were considered in the present study.

Informed consent The authors of this manuscript accept that the paper is submitted for publication in the Journal of Pest Science and report that this paper has not been published or accepted for publication in another journal, nor is under consider at another journal.

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