# "To Eat, or Not to Eat, That is the Question" - Answered by Real-Time Monitoring Techniques Combining with Computational Analysis for Feeding Behavior Study of Crapemyrtle Bark Scale (*Acanthococcus lagerstroemiae*)

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**Summary**

Crapemyrtle bark scale [(CMBS) *Acanthococcus lagerstroemiae*], an invasive and polyphagous sap feeder, has spread across 17 U.S. states. The infestation of CMBS negatively impacts the flowering and fruiting of various ornamental and fruit plants. Crapemyrtle bark scale host confirmation is critical to determine the insect's potential risks to the Green Industry and help develop strategic management of CMBS. Previously confirming CMBS hosts was time-consuming. We investigated the CMBS feeding activities using the electrical penetration graph (EPG) to monitor real-time stylet penetration to determine potential hosts more efficiently. First, we characterized typical EPG waveforms (waveform C, waveform potential drop, the total

duration of E1 and E2, and total duration of waveform G) of feeding activities for CMBS on a validated host, *Lagerstroemia limii*. We then tested the feeding behavior of CMBS using different species, including *L. speciosa*, *L. indica* × *speciosa* '18096', Mexican beautyberry (*Callicarpa acuminata*), three Ficus species (*F. pumila*, *F. tikoua*, and *F. auriculata*), and soybean (*Glycine max*), with the positive control (*L. limii*). Results showed that plant species significantly impacted phloem sap ingestion of CMBS, which could be used to rapidly confirm a a potential CMBS host.

**INTRODUCTION**

Crapemyrtle bark scale (CMBS), *Acanthococcus lagerstroemiae* (Hemiptera: Eriococcidae), is an invasive polyphagous insect (Kozár et al., 2013) which has spread across 17 U.S. states since its initial report in Texas in 2004 (EDDMapS, 2021). The reduction in flowering or fruiting on ornamental plants and crops resulted from the infestation and the observation of CMBS found on native species sharpened the concern about this invasive insect's threat potential to the Green Industry and ecosystems (Gu et al., 2014; Merchant et al., 2018; Wu et al., 2021; Xie et al., 2020; Zhang and Shi, 1986). Therefore, it would be crucial to determine the host range of this relatively new invasive insect posed to the local economy and ecosystem.

The host range assessment involves accepting or rejecting plant species via insect feeding performance (Schoonhoven et al., 2005). However, measuring the feeding performance of sap-sucking insects typically needs time-consuming tests regarding biological traits (Herbert et al., 2009; Wang et al., 2019; Wu et al., 2021; Wu et al., 2010). Stylet penetration could be a vital parameter for sap feeders to rapidly assess the host range through a real-time feeding monitor technique and an electrical penetration graph [EPG (Prado and Tjallingii, 1997)]. The EPG technique can track the position of the hemipterans' stylet tips in different plant tissue via

voltage fluctuations amplified as specific EPG waveforms (Tjallingii, 1985), and these EPG waveforms were associated with biological feeding activities through histology correlation work (Tjallingii and Esch, 1993). Applying the EPG monitoring techniques in the feeding behavior study of CMBS could confirm the host rapidly and improve the understanding of the CMBS-plant interaction, which would help develop integrated management of CMBS.

To date, little is known about the feeding behavior of CMBS or the CMBS-plant interaction. This study aimed to characterize EPG waveforms related to the feeding activities of CMBS on a validated host plant (*Lagerstroemia limii*) and compare feeding activities among different plant species to assess the plant host suitability for CMBS rapidly.

## MATERIALS AND METHODS

**Insects and Plants.** Colonies of CMBS were established by attaching CMBS-infested branches to healthy *L. limii* plants and maintained in a handmade chiffon mesh-covered cage (58.0 cm long × 58.0 cm wide × 50.0 cm high) in a CONVIRON® (Controlled Environments Ltd., Winnipeg, Manitoba, Canada) growth chamber [25 ±1 °C, 60±5 % relative humidity (RH), and a photoperiod of 16 h light (L):8 h dark (D)] at the Department of Biology, Texas A&M University. All CMBS used for EPG recordings were female adults of CMBS (2.1 ± 0.7 mm long; 1.2 ± 0.5 mm wide) obtained from the colony.

Plants used for characterizing the feeding behavior of CMBS were validated host plant *L. limii* (n = 20). Plants used for comparing the feeding behavior by plant species were *L. limii* (n = 25), *Lagerstroemia speciosa* (n = 25), *Lagerstroemia indica* × *speciosa* '18096' (n = 20), *Callicarpa acuminata* (n = 20), *F. auriculata* (n = 20), *Ficus pumila* (n = 20), *Ficus tikoua* (n = 20), and *Glycine max* (n = 25). The Arabic number in the paratheses represented how many plants were tested for each species. The crapemyrtle plants (*L. limii* and *L. speciosa*) were

initially provided by North Florida Research and Education Center (Quincy, FL). The crapemyrtle hybrid '18096' was selected from our crapemyrtle breeding program at the Department of Horticultural Sciences (College Station, TX). The *Ficus* species and Mexican beautyberry (*C. acuminata*) were initially provided by John Fairey Garden Conservation Foundation (Hempstead, TX). All these test plants were propagated via cuttings. They were maintained in 1 qt plastic pots (The HC Companies, Twinsburg, OH) filled with Jolly Gardener Pro-Line C/25 growing mixture (Oldcastle Lawn and Garden Inc, Poland Spring, ME) in the greenhouse at 25 ± 5 ℃, 50 ± 10% RH, and a photoperiod of 10.5:13.5 (L:D) h.

**Electrical penetration graph recordings of CMBS feeding on different plant species.** The CMBS penetration activities were monitored by the EPG devices on different plant species, using individual CMBS female adults in a Faraday cage to characterize the feeding behavior of CMBS and test if plant species affect the feeding behavior. The EPG experiment was conducted in a climate-controlled room (25 ±1 ℃, 60±5 % RH, and a 16 h: 8h photoperiod) at the Department of Biology. The feeding behavior was monitored and recorded for 24 hours, and the recording was replicated using a new insect and a new plant for each species per time.

All typical EPG waveforms in the recordings were labeled manually. After comparing with histological studies and EPG waveforms on other sap-sucking insects (Prado and Tjallingii, 1994; Tjallingii, 1985; Tjallingii and Esch, 1993; Tjallingii, 2006), typical feeding waveforms for CMBS on the host were characterized by visually identification using the EPGminer. Based on the biological feeding activities, EPG parameters about the feeding activities of CMBS on each plant species were considered, including the total duration of pathway phase (waveform C and waveform potential drop), the total duration of E1, the total duration of phloem sap ingestion (waveform E2), and the total duration of waveform G.

**Data processing and statistical analysis.** The ggplot2 (Hadley, 2016) and the plotly (Sievert, 2020) were used to generate visuals from R. The epgminer package (supplementary) was newly developed to extract and analyze the EPG data. The values for the frequency, duration, occurrence, and voltage (mean, standard deviation, and relative amplitude) were calculated using epgminer function, wave_topfreq, wave_occurrence, and wave_volts, respectively (Supplementary).

Data analysis was performed using JMP® 16 (SAS Institute, Cary, NC). The parameters listed in Table 1 were analyzed using the one-way analysis of variance (ANOVA) to test the effect of plant species on the total duration of each feeding waveform. Tukey's Honestly Significant Difference (HSD) test ($\alpha$ =0.05) was used to compare the difference in each mean value.

## RESULTS AND DISCUSSION

**Characterization of typical EPG waveforms for CMBS feeding behavior.** EPG signals were characterized for CMBS when feeding on a host plant *L. limii* (Table 1), according to their shape, voltage level (extra- or intracellular), relative amplitude, frequency, and duration. These waveforms were labeled as C, pd1, pd2, E1, E2, and G.

Waveform C (Fig. 1A), correlating to gel salivation and other stylet pathway activities, was detected whenever CMBS started penetration and intercellular stylet pathway. Potential drops (Fig. 1B) were frequently observed during the stylet pathway phase. At the start, the voltage suddenly dropped when the stylet was supposed to puncture cells; during the low intracellular voltage level, the potential drops were often clearly divided into potential drop 1 (pd 1) and potential drop 2 (pd 2) periods. Waveform E complex (Fig. 1B), consisting of E1 and E2 phases during phloem phase, often sequentially followed the stylet pathway phase. The voltage level of

the complex gradually and dramatically dropped below zero, which was much lower than other waveforms. Waveform E1, correlated to watery salivation, had positive peaks. Waveform G (Fig. 1C), correlated to xylem sap ingestion during the xylem phase, had a higher voltage level (extracellular) than other waveforms.

**Comparison of feeding parameters of CMBS among different plant species.** Even though plant species did not affect the total duration of waveform E1 ($F = 1.9326$; df = 7, 71; $P = 0.0769$), it affected the total duration of waveform C ($F = 6.8815$; df = 7, 71; $P < 0.0001$) and the total duration of waveform E2 ($F = 8.2204$; df = 7, 71; $P < 0.0001$) [Table 2]. After reaching the sieve elements, the insect spent the longest time in phloem sap ingestion on *L. limii* (234.78 ± 60.16 min) and the crapemrytle hybrid '18096' (286.43 ± 136.38 min), which was at least twice longer duration on *L. speciosa* (85.49 ± 38.84 min) and *C. acuminata* (19.84 ± 6.48 min). No individuals had phloem salivation or phloem ingestion on *F. pumila*, *F. auriculata*, or *G. max*.

Comparing with *F. auriculata*, even though the total duration of waveform G was multiple times greater on other species where the xylem ingestion occurred (varying from 90.27 ± 57.43 min to 423.54 ± 88.01 min), no significance was shown among the species [*L. limii*, *L. speciosa,* the hybrid '18096', *C. acuminata*, *F. tikuoa*, *F. pumila*, and *G. max* ($F = 1.8371$; df = 7, 71; $P = 0.0934$)].

From the perspective of stylet penetration activities, our study is the first report to elucidate the occurrences of phloem and xylem ingestion by CMBS on its host plant through the EPG techniques. We developed an R-programming-based application to help identify and characterize the EPG waveforms with less human input. The comparison results of feeding waveforms among different species indicated that CMBS accomplished the ingestion of phloem sap and xylem sap on validated host plants (*C. acuminata*, *F. tikoua, L. limii*, *L. speciosa*) and the crapemyrtle

hybrid ‘18096’. But CMBS did not intake phloem sap on *F. pumila*, *F. auriculata,* and *G. max*. With that, “To eat, or not to eat, that is the question.” This was answered by the application of the EPG techniques combining with computational analysis in feeding behavior study of CMBS.

**SUPPLEMENTARY**

1) algorithm: https://github.com/LylChun/epgminer

2) EPGminer in website version: https://epgdata.shinyapps.io/epgminer_app/

and software version:

https://github.com/LylChun/epgminer/tree/master/inst/epgminer_app/rsconnect/shinyapps.io/epgdata

**Literature Cited**

EDDMapS. 2021. Early Detection & Distribution Mapping System. 15 March 2021. < https://www.eddmaps.org/distribution/viewmap.cfm?sub=80722>.

Gu, M., Merchant, M., Robbins, J. and Hopkins, J. (2014). Crape myrtle bark scale: A new exotic pest. EHT-049, 3. 11 May 2019. <https://cdn-ext.agnet.tamu.edu/wp-content/uploads/2018/10/EHT-049-crape-myrtle-bark-scale-a-new-exotic-pest.pdf>.

Hadley, W. (2016). Ggplot2: Elegrant graphics for data analysis. Springer.

Herbert, J.J., Mizell III, R., and McAuslane, H. (2009). Host preference of the crapemyrtle aphid (Hemiptera: Aphididae) and host suitability of crapemyrtle cultivars. Environ. Entomol., *38*:1155-1160.

Kozár, F., Kaydan, M.B., Konczné Benedicty, Z., and Szita, E. (2013). Acanthococcidae and related families of the Palaearctic Region. Plant Protection Institute, Centre for Agricultural Research, Hungarian, Budapest, Hungary.

Merchant, M., Garcia, J., and Gu, M. (2018). Longevity of imidacloprid as a soil treatment for crapemyrtle bark scale on crapemyrtle, 2016–2017. Arthropod Management Tests, 43, tsy012.

Prado, E. and Tjallingii, W.F. (1994). Aphid activities during sieve element punctures. Entomol. Experim. Applic. *72:* 157-165.

Prado, E. and Tjallingii, W.F. (1997). Effects of previous plant infestation on sieve element acceptance by two aphids. Entomol. Experim. Applic., *82:* 189-200.

Schoonhoven, L.M., Van Loon, B., van Loon, J.J., and Dicke, M. (2005). Insect-plant biology. Oxford University Press on Demand.

Sievert, C. (2020). Interactive web-based data visualization with R, plotly, and shiny. CRC Press.

Tjallingii, W. (1985). Electrical nature of recorded signals during stylet penetration by aphids. Entomol. Experim. Applic., *38:* 177-186.

Tjallingii, W. and Esch, T.H. (1993). Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. Physiol. Entomol. *18:* 317-328.

Tjallingii, W.F. (2006). Salivary secretions by aphids interacting with proteins of phloem wound responses. J. Exper. Bot. *57:* 739-745.

Wang, Z., Chen, Y., and Diaz, R. (2019). Temperature-dependent development and host range of crapemyrtle bark scale, *Acanthococcus lagerstroemiae* (Kuwana) (Hemiptera: Eriococcidae). Fla. Entomol., *102:* 181-186.

Wu, B., Xie, R., Knox, G.W., Qin, H., and Gu, M. (2021). Host suitability for crapemyrtle bark scale (*Acanthococcus lagerstroemiae*) differed significantly among crapemyrtle species. Insects, *12:* 6. doi: 10.3390/insects12010006

Wu, X.-H., Zhou, X.-R., and Pang, B.-P. (2010). Influence of five host plants of *Aphis gossypii* Glover on some population parameters of *Hippodamia variegata* (Goeze). J. Pest Sci. *83:* 77-83.

Xie, R., Wu, B., Dou, H., Liu, C., Knox, G.W., Qin, H., and Gu, M. (2020). Feeding preference of crapemyrtle bark scale (*Acanthococcus lagerstroemiae*) on different species. Insects*, 11:* 399. doi: 10.3390/insects11070399.

Zhang, Z. and Shi, Y. (1986). Studies on the morphology and biology of *Eriococcus lagerstroemiae* kuwana. J. Anhui Agric. Univ., 2. 28 March 2020. <https://en.cnki.com.cn/Article_en/CJFDTotal-SCHO198602011.htm>.

**Table 1**. Characteristics of the EPG waveforms recorded during CMBS feeding on *Lagerstroemia limii.*

| EPG waveform | | Waveform characteristics | | | | Correlations |
|---|---|---|---|---|---|---|
| | | Voltage level | Frequency (Hz) | | Relative amplitude (%)[Z] | Activities assigned for similar waveforms in other hemipterans |
| | | | Min-Max | Medium ± SE | | |
| C | | Extracellular | 0.59-1.61 | 0.98 ± 0.10 | 11.81± 1.00 | Sheath salivation and other intercellular stylet pathways |
| pd | pd1 | Intracellular | 0.42-6.10 | 4.35 ± 0.71 | 20.20 ± 2.20 | Short cell punctures |
| | pd2 | Intracellular | 1.25-3.71 | 3.07 ± 0.28 | 23.38 ± 2.70 | |
| E1 | | Intracellular | 0.49-2.05 | 1.08 ± 0.24 | 32.43 ± 1.80 | Phloem salivation |
| E2 | | Intracellular | 0.49-2.05 | 0.78 ± 0.20 | 34.53 ± 2.90 | Phloem sap ingestion |
| G | | Extracellular | 1.37-3.00 | 1.86 ± 0.20 | 11.72 ± 0.30 | Xylem sap ingestion |

[Z] Relative amplitude (%) = (mean of amplitude for each waveform - mean of amplitude for non-probing)/ 5 ×100%

C represents the stylet pathway phase; E1 represents phloem salivation; E2 represents phloem ingestion; G represents xylem ingestion.

**Table 2** Electrical penetration graph parameters of CMBS feeding on different plant species.

| Electrical penetration graph parameter | Plant Type | | | | | | | | *P*-value |
|---|---|---|---|---|---|---|---|---|---|
| | ***Lagerstroemia limii*** | ***L. speciosa*** | ***Lagerstroemia indica* × *speciosa* '18096'** | ***Callicarpa acuminata*** | ***Ficus tikoua*** | ***Ficus pumila*** | ***Ficus auriculata*** | ***Glycine max*** | |
| 1. Total duration of C (min) | 488.65 ± 84.53 bc | 428.32 ± 76.55 bc | 671.19 ± 216.54 abc | 549.78 ± 86.02 bc | 768.57 ± 54.24 ab | 477.63 ±108.22 bc | 1183.10 ± 153.45 a | 262.20 ± 57.31 c | < 0.0001 |
| 2. Total duration of pd (min) | 14.76 ± 3.25 ab | 24.34 ± 7.00 ab | 19.87 ± 4.54 ab | 26.20 ± 9.25 ab | 43.90 ± 12.82 a | 20.87 ± 7.23 ab | 14.77 ± 11.76 ab | 3.17 ± 1.74 b | 0.0128 |
| 3. Total duration of E1 (min) | 63.33 ± 32.46 a | 35.37 ± 11.37 a | 51.36 ± 19.15 a | 47.90 ± 11.77 a | 39.64 ± 9.75 a | 0.00 a | 0.00 a | 0.00 a | 0.0769 |
| 4. Total duration of E2 (min) | 234.78 ± 60.16 a | 85.49 ± 38.84 bc | 286.43 ± 136.38 ab | 19.84 ± 6.48 c | 99.66 ± 26.79 abc | 0.00 c | 0.00 c | 0.00 c | < 0.0001 |
| 5. Total duration of G | 288.52 ± 54.60 a | 239.28 ± 86.96 a | 90.27 ± 57.43 a | 289.15 ± 64.38 a | 317.46 ± 39.78 a | 423.54 ± 88.01 a | 0.00 a | 190.79 ± 79.39 a | 0.0934 |

C represents the stylet pathway phase; E1 represents phloem salivation; E2 represents phloem ingestion; G represents xylem ingestion.

Means (± SE) followed by different letters within a row are different by Tukey's Honestly Significant Difference (HSD) test ($\alpha$ =0.05).

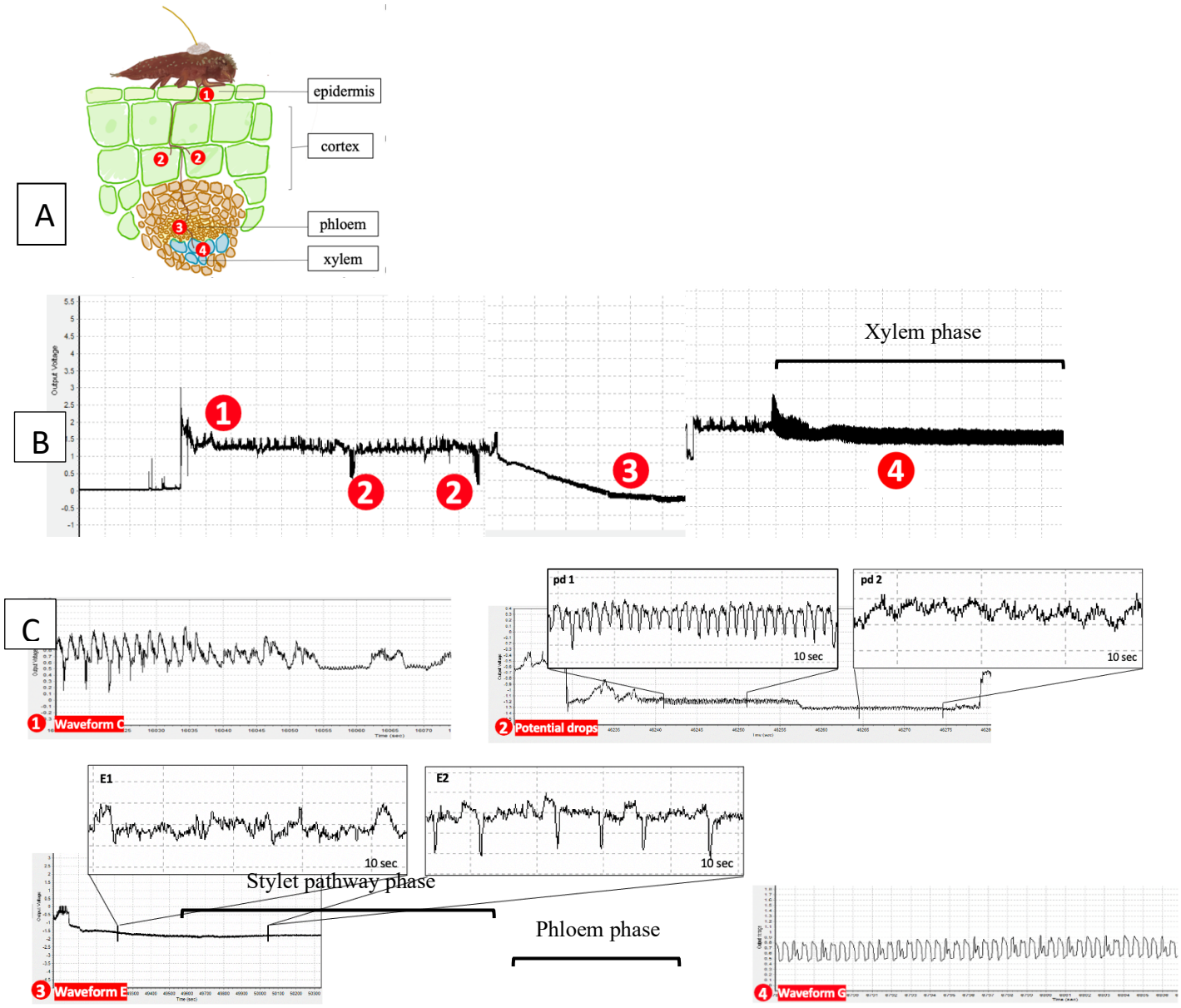


**Figure 1.** General scheme of characteristic feeding behavior of CMBS on *Lagerstroemia limii.* A: A diagram shows CMBS's stylet tip positions in a plant's stem when feeding. B: General scheme of characteristic EPG waveforms. C: ① Waveform C was detected when CMBS was probing intercellular part; ② Waveform potential drop (pd) was detected when the stylet tip punctured plant cells; ③ Waveform E1 was detected when intracellular stylet activity in mesophyll and phloem salivation occurred; Waveform E2, characterized by negative peaks, was detected when phloem sap ingestion occurred; ④ Waveform G was detected when xylem sap

ingestion occurred. [C represents the stylet pathway phase; E1 represents phloem salivation; E2 represents phloem ingestion; G represents xylem ingestion].