Short Communication

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# Detecting Ingested Host Plant DNA in Potato Leafhopper (Hemiptera: Cicadellidae): Potential Use of Molecular Markers for Gut Content Analysis

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## Abstract

Detection of host plant DNA from sap-feeding insects can be challenging due to potential low concentration of ingested plant DNA. Although a few previous studies have demonstrated the possibility of detecting various fragments of plant DNA from some sap-feeders, there are no protocols available for potato leafhopper, *Empoasca fabae* (Harris) (Hemiptera: Cicadellidae), a significant agricultural pest. In this study we focused on optimizing a DNA-based method for host plant identification of *E. fabae* and investigating the longevity of the ingested plant DNA as one of the potential applications of the protocol. We largely utilized and modified our previously developed PCR-based method for detecting host plant DNA from grasshopper and the spotted lanternfly gut contents. We have demonstrated that the *trn*L (UAA) gene can be successfully utilized for detecting ingested host plant DNA from *E. fabae* and determining plant DNA longevity. The developed protocol is a relatively quick and low-cost method for detecting plant DNA from *E. fabae*. It has a number of important applications—from determining host plants and dispersal of *E. fabae* to developing effective pest management strategies.

**Key words:** *Empoasca*, host plant DNA, insect–plant interactions, molecular gut content analysis, sap-feeding insects

Potato leafhopper, *Empoasca fabae* (Harris) (Hemiptera: Cicadellidae), is a sap-feeding insect and a wide-spread agricultural pest in the United States and Canada which causes substantial plant damage (hopperburn) triggered by a plant wound response (Backus et al. 2005, DeLay et al. 2012). The leafhopper is highly polyphagous, with over 220 known reproductive host plant species in 26 plant families (Lamp et al. 1994). While adults often occur on other plant species, whether *E. fabae* feeds on nonreproductive plants or uses them for resting only is poorly understood.

Use of molecular markers has many advantages for understanding plant usage by various insects (García-Robledo et al. 2013, Avanesyan and Lamp 2020). Molecular gut content analysis of *E. fabae* would help us accurately identify host plants and may predict a potential host switch. To date, however, to the best of our knowledge, no studies have been conducted on detection of ingested host plant DNA from *E. fabae*. Previous studies that involved molecular diet analysis were primarily conducted on leaf-chewing insects, such as beetles (Jurado-Rivera et al. 2009, Wallinger et al. 2013), moths (Miller et al. 2006), and orthopterans (Avanesyan and Culley 2015, Avanesyan 2014). Sap-feeding insects, such as *E. fabae*, are more challenging for molecular analysis of their gut contents because phloem sap presumably does not contain plant DNA (Avanesyan and Lamp 2020). However, such gut content analysis was shown to be helpful for tracking the landscape movements of psyllid species (Cooper et al. 2019), as well as for determining host usage of the spotted lanternfly (Avanesyan and Lamp 2020). Pearson et al. (2014) suggested in their study with psyllids that psyllids' stylet could sample parenchyma cells while locating the phloem tubes; this could explain DNA detection in the guts of sap-feeding insects.

To address these issues for *E. fabae*, we focused on the following objectives: 1) to develop an effective protocol for detection of host plant DNA in *E. fabae*; and 2) to explore the longevity of the host plant DNA within the insect body, as one of the potential applications of this protocol (Fig. 1). Based on our recent findings from molecular gut content analysis of another sap-feeder, *Lycorma delicatula* (White) (Hemiptera: Fulgoridae) (Avanesyan and Lamp 2020), we expected that the ingested plant DNA is detectable in *E. fabae*. However, due to a smaller size of *E. fabae* body (compared to that of *L. delicatula*), we expected ingested plant DNA to be at very low concentration and show a weaker signal during gel electrophoresis.

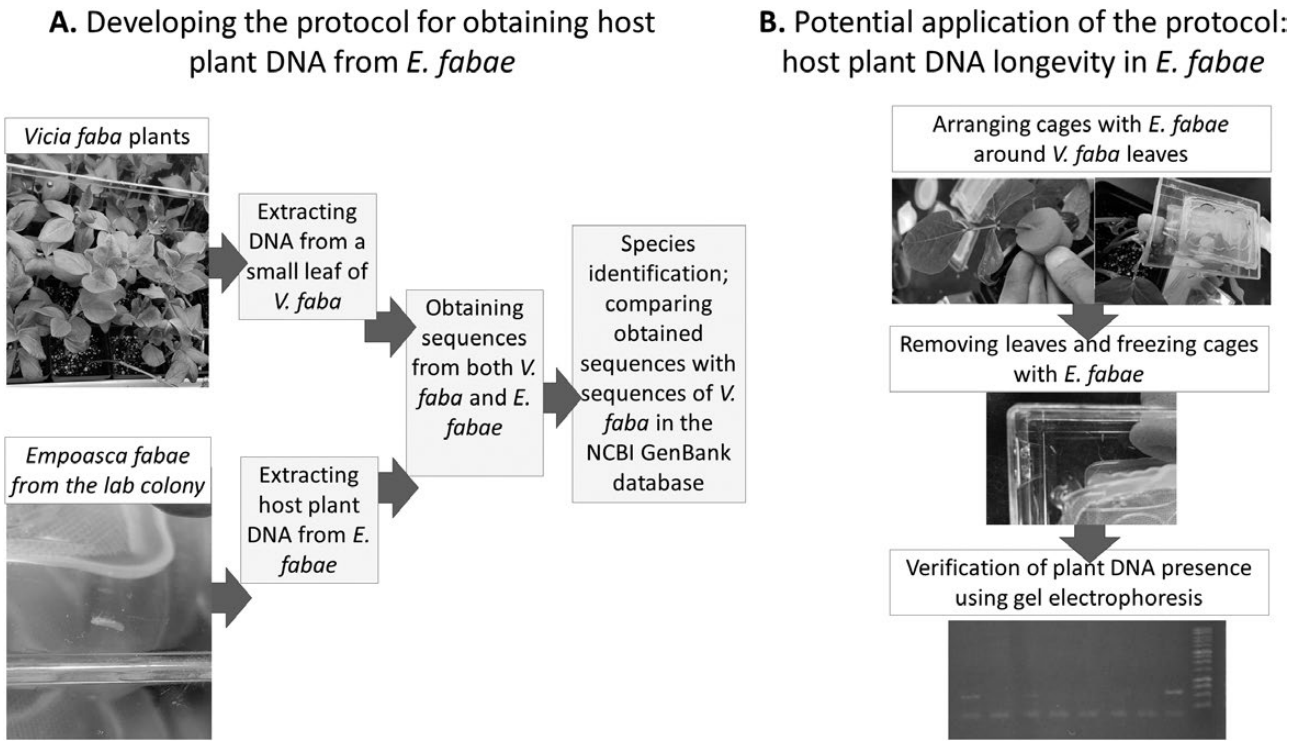


**Fig. 1.** The overview of the experimental setup. (A) Developing the protocol for obtaining DNA of *Vicia faba* plants from the potato leafhopper, *Empoasca fabae*; main steps from obtaining the plant and insect samples to sequence analysis. (B) Potential application of the protocol: exploring plant DNA longevity in *E. fabae*. The obtained sequences of intact and ingested *V. faba* are deposited to NCBI GenBank (accession no.: MK934667, MK837073) (Photos by A. Avanesyan).

## Materials and Methods

The fava bean plants (*Vicia faba* (L.) (Fabales: Fabaceae)), which serve as a host plant species for *E. fabae*, were grown from seed in the Research Plant Growth Facility of the University of Maryland from May to October 2018. Two- and three-week-old potted plants, approximately 20–25 cm height, were used for maintaining a lab colony of *E. fabae* which were initially collected at the Western Maryland Research and Education Center (Keedysville, MD). For the purpose of this study, only adults were used for detection of ingested plant DNA.

### Detecting DNA of Vicia faba From the Gut Contents of Empoasca fabae

For this study, we largely utilized our previously developed PCR-based method for detecting a portion of plant chloroplast gene (*trn*L (UAA)) from grasshopper gut contents (Avanesyan 2014), as well as recent modifications of that protocol for *L. delicatula* (Avanesyan and Lamp 2020). In total, 20 adults of *E. fabae* were randomly collected from the culture, and then were immediately frozen at –20°C along with a small leaf from fava bean plants which was used later as a positive control for PCR amplification. Following the steps described in Avanesyan and Lamp (2020), the whole body of an insect (submersed in 2% bleach solution for 1 min) and a leaf from fava bean plants were utilized for DNA extraction (DNeasy blood and tissue kit, Qiagen Inc., Germantown, MD). Rinsing the insects with bleach solution is commonly used to sterilize insect body surface from any plant remains and to ensure that all the detected plant DNA is the ingested plant DNA (Cooper et al. 2016, 2019; Diepenbrock et al. 2018; Avanesyan and Lamp 2020). Following these studies, we expected that all the detected DNA from *V. faba* would be presumably ingested by *E. fabae*.

Samples were then stored at –4°C until they were used for PCR amplification. A partial chloroplast *trn*L (UAA) gene (~500 bp) was amplified using primers *trn*Lc-*trn*Ld (Taberlet et al. 1991) purchased from Integrated DNA Technologies (Coralville, IA) under PCR conditions described in Avanesyan and Lamp (2020). Relative DNA template concentration was quantified using the Invitrogen Qubit 4 Fluorometer. The presence of the DNA template was verified using 1% agarose gel. PCR products were then purified using ExoSAP-IT (Affymetrix Inc., Santa Clara, CA), and sequenced using Sanger sequencing at GENEWIZ (GENEWIZ Inc., South Plainfield, NJ). The obtained DNA sequences from both the plant and insect samples were trimmed and aligned using BioEdit (Hall 1999). Plant species identity was determined using BLAST (the NCBI GenBank database; http://www.ncbi.nlm.nih.gov/genbank/).

### Potential Application of the Protocol: Longevity of Ingested Plant DNA

To demonstrate how the developed protocol can be applied for exploring plant DNA longevity in *E. fabae*, a separate feeding experiment was conducted. Ten pots with fava bean plants were selected; one small plastic cage, 12 × 9 × 3.5 cm, per plant was then set up around the top part of plant stems following Avanesyan et al. (2019). Three–five adult leafhoppers were introduced in each cage where they were allowed to feed on the enclosed leaves for 24 h. After feeding, the leaves were removed from the cages, and the cages were checked for the number of live leafhoppers. Then, the cages with leafhoppers were frozen at –20°C at 10 time intervals: 30 min, 45 min, 1, 2, 5, 6, 7, 8, 18, and 20 h post-ingestion. In this experiment, we did not aim to explore all the time points

but rather to show the application of the developed protocol to investigate the plant DNA longevity in *E. fabae*. Consequently, for the purpose of the study, plant DNA was then extracted from 1–3 insect individuals per time interval (i.e., per cage) following the procedure described above; the presence of DNA was verified by gel electrophoresis. Intensity of the DNA signal was compared to that of the positive control (i.e., PCR product obtained from DNA from an intact fava bean plant, which showed the strongest signal on the gel image), and was described as: 1) strong (similar to control); 2) relatively strong (~75% of the signal intensity of the control); 3) weak (~50% of the signal intensity of the control); 4) very weak (a pale band with ~25% of the signal intensity of the control); and 5) none (no signal).

## Results

### Detecting DNA of Vicia faba From the Gut Contents of Empoasca fabae

Our developed PCR protocol demonstrated that plant DNA can be reliably detected in *E. fabae*: the gel electrophoresis confirmed the presence of the ingested plant DNA in all 20 samples. All the DNA 'bands' had relatively strong intensity compared to that in the positive control. The relative concentration of the DNA template in purified PCR products was 0.061 ± 0.01 ng/µl ($n$ = 10). Sequence analysis of the purified PCR products revealed that primers *trn*Lc and *trn*Ld successfully amplified a 496-bp region of the chloroplast *trn*L (UAA) gene from experimental *V. faba* plant and a 511-bp region of the chloroplast *trn*L (UAA) gene from *E. fabae*. The obtained sequences showed 100% identity between the plant and insect samples, and 99% identity (with *E*-value = 0) with *V. faba* sequence retrieved from the NCBI GenBank database. The sequences of *V. faba*, isolated from both plant and insect samples, are deposited at the NCBI GenBank database (accession no.: MK934667, MK837073).

### Potential Application of the Method: Longevity of Ingested Plant DNA

In total, 10 adult females of *E. fabae* were used to test the presence of ingested plant DNA at different time intervals post-ingestion, with 1–3 adults per time point. Our results demonstrated the presence of plant DNA in *E. fabae* individuals during all the time points. Interestingly, despite our expectation that the DNA signal would fade over time, the intensity of the DNA signal did not seem to follow any pattern. We detected various intensities of the DNA signal at different time points: from very weak to relatively strong. Relatively strong DNA signals were observed at 45 min post-ingestion, as well as at 2, 5, 6, 7, 8, and 18 h post-ingestion; weak DNA signals were recorded at 1 and 20 h post-ingestion; and a very weak signal was recorded at 30 min post-ingestion only.

## Discussion

The choice of the targeted plant DNA locus is one of the primary factors which could affect the detectability and identification of ingested plant DNA in *E. fabae*. The region of P6 loop of the *trn*L intron, which we used in our study, has been reported to be less sensitive to amplification of degraded DNA but can show low species resolution (Valentini et al. 2009). In our recent study on *L. delicatula* we demonstrated the amplification success of *rbc*L gene (Avanesyan and Lamp 2020). Future studies might focus on utilization of other loci for host plant identification on species level, such as the nuclear ribosomal DNA or the internal transcribed spacers or use a combination of loci (Kress et al. 2009).

The feeding mechanism which *E. fabae* utilizes on different host plants may also contribute to the intensity of the plant DNA signal. It has been reported that the amount of phloem feeding versus laceration-style mesophyll feeding which *E. fabae* performs on different host plants may differ (Backus and Hunter 1989). We hypothesize that the DNA from the host plant which *E. fabae* utilizes for mesophyll feeding is easier to detect compared with phloem feeding due to the presence of the plant cell components in the insect guts. Differences in the probe location (i.e., the place on a plant into which the insect inserts its stylets) and probe duration (i.e., how long the insect keeps its stylets inserted) between and within host plants (Backus and Hunter 1989) may also indicate differences in the amount of plant material consumed. This, in turn, may explain differences in the DNA signal intensity we detected over time.

The detectability of plant DNA in *E. fabae* in 20 h post-ingestion was somewhat surprising for a relatively small sap-feeding insect; however, based on previous studies and our work with other insects, we expected to detect the plant DNA during at least first 2–3 h post-ingestion. Previously, we were able to detect the ingested plant DNA in the differential grasshopper, *Melanoplus differentialis* (Thomas) (Orthoptera: Acrididae) up to 22 h post-ingestion (Avanesyan 2014). Diepenbrock et al. (2018) in their experiments with *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) and strawberry plants showed that the host plant DNA can be detected in *D. suzukii* gut contents for up to 7 d post-ingestion. For future studies, it would be helpful to investigate differences in gut morphology between leaf-grazing and sap-feeding insects and its effect on plant DNA detectability, as well as the plant DNA detection rate in *E. fabae* after transferring insects to a new host plant.

Additionally, future studies might explore the ingested content in sap-feeders, particularly the presence of pathogens, which was beyond the focus of our study. It has been shown, for example, that the meadow spittlebug, *Philaenus spumarius* (L.) (Hemiptera: Aphrophoridae), a vector of *Xylella fastidiosa* (Wells et al.) (Xanthomonadales: Xanthomonadaceae), can ingest this bacterium with plant sap, and this pathogen can be detected in insect foregut (but not in the other parts of *P. spumarius* digestive system) (Cornara et al. 2017). Finally, incorporating additional steps in our developed approach, such as sequencing of the cloned amplicons (e.g., Wang et al. 2018), or using high-throughput sequencing (e.g., Cooper et al. 2019), will be invaluable for tracking the dispersal of *E. fabae* among different crop plants, as well as *E. fabae* feeding prior to its migration to the agricultural fields.

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