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Short Communication

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Detecting Specific Resource Use by *Drosophila suzukii* (Diptera: Drosophilidae) Using Gut Content Analysis

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Abstract

Drosophila suzukii Matsumura (Diptera: Drosophilidae) is an invasive, highly polyphagous pest of soft-skinned fruits throughout much of the world. A better understanding of the ecology of adult flies, including their nutritional resources, is needed to advance ecologically based management approaches. In this study, we evaluate the capability of polymerase chain reaction-based gut content analysis to detect a known food resource from DNA extracted from laboratory-reared flies. Using strawberry as a focal host and available DNA primers, we validated that DNA from this host could be detected for up to 7 d post-consumption. With the development of specific primers for additional hosts, we expect that this technique will enable researchers to better understand how *D. suzukii* adults use, and move between, nutritional resources.

Key words: spotted wing drosophila, gut content, plant pest

Drosophila suzukii Matsumura (Diptera: Drosophilidae), spotted wing drosophila, is a polyphagous invasive pest of soft-skinned fruits, which has quickly spread throughout much of the world (reviewed in Asplen et al. 2015). *D. suzukii* is found in all fruit growing areas of the United States and causes significant yield loss in cultivated host crops including blueberries, cherries, caneberries (blackberries and raspberries), grapes, and strawberries (Bolda et al. 2010, Goodhue et al. 2011, Burrack et al. 2013). In the United States, potential annual yield loss has been estimated at over $700 million (Asplen et al. 2015).

Due to the economic impact of *D. suzukii*, early research has focused largely on identifying infestation control measures, including insecticide rotations (Beers et al. 2011; Van Timmeren and Isaacs 2013; Diepenbrock et al. 2016a, 2017), harvest frequency (Leach et al. 2017), and post-harvest cold storage (Aly et al. 2017).

Recent studies have described *D. suzukii*'s wide host range, including both crop and noncrop fruit (Lee et al. 2015, Poyet et al. 2015), have attempted to document the movement of *D. suzukii* between natural habitats and commercial host crops (Klick et al. 2014, Pelton et al. 2016, Swoboda Bhattarai 2017), and addressed the role of noncrop hosts in the survival of *D. suzukii* in the absence of crop resources (Diepenbrock et al. 2016b). These studies have relied upon captures in baited (Pelton et al. 2016, Swoboda Bhattarai 2017) or passive (Swoboda Bhattarai 2017) traps or indirect measures of habitat association using mark-recapture methods (Klick et al. 2014). Gut content analysis, frequently used to study interactions between predators and prey (e.g., King et al. 2008, Lundgren and Fergen 2011), could be applied to describe the feeding associations of adult *D. suzukii*. This tool has yet to be widely used for the study of plant-feeding insect pests (discussed in Wallinger et al. 2013). In this study, we 1) explore the potential to use gut content analysis to detect a known food resource, strawberry, within *D. suzukii* and 2) determine the duration of detection of this resource post-consumption under controlled conditions.

Materials and Methods

Source Material

A colony of *D. suzukii* has been maintained for over 50 generations within a laboratory rearing facility at North Carolina State University. Original flies were collected from blackberries and raspberries at the Upper Mountain Research Station, Laurel Springs, NC, in October 2010. *D. suzukii* are maintained on a standard cornmeal *Drosophila* diet and held at 20°C with a 16:8 (L:D) h cycle (Burrack et al. 2013). Wild flies are periodically added to the colony to increase genetic diversity. All flies used in experiments were 5- to 7-d-old females at the time of feeding.

Strawberries, *Fragaria* × *ananassa* Duch., cv. 'Albion' (Shaw and Larson 2006), were collected at a farm in Cleveland County, NC (April 2016) and frozen until use in experiments. Blueberries, blackberries, and raspberries were purchased (Fall 2016), inspected for *D. suzukii* eggs, and frozen prior to preparation. To feed flies, fruit were thawed and pureed.

Feeding Methods

D. suzukii were starved for 24 h with access to a water-moistened cotton ball. Feeding arenas consisted of 473-ml plastic cups (PFS Sales Co., Raleigh, NC) sealed with cellophane wrap (Glad ClingWrap, Oakland, CA) with a 5-cm white tulle mesh-covered vent on one side to allow airflow (Fig. 1A). Each arena was provisioned with an open 35- × 10-mm Petri dish (Falcon, #351008) containing 1 ml of one type of pureed fruit for feeding (e.g., strawberry) with 0.01 g of table sugar to induce feeding. Cohorts of 25 flies were introduced into arenas and allowed to feed for 2 h (Fig. 1B).

After 2 h, flies were observed for visual confirmation of feeding using a stereomicroscope (Fig. 1C). This was repeated until at least 200 fed flies were obtained. Flies were then either 1) placed in a 50-ml Falcon tube capped with a moistened cotton ball with no additional food resources and sacrificed via freezing every 2 h for 24 h or 2) held on a cornmeal-based fly diet after initial feeding on strawberry to detect the presence of host DNA at 48, 120, and 168 h after feeding. Control flies were sacrificed immediately after 24-h starvation period and held in 70% EtOH until DNA was extracted. Quality genomic DNA can be extracted for up to 168 h from flies trapped and preserved in propylene glycol in a field setting (A. Abrieux and J. Chiu, personal communication). After freezing, flies were transferred to 70% ethanol (Weber and Lundgren 2009) and held at –20°C.

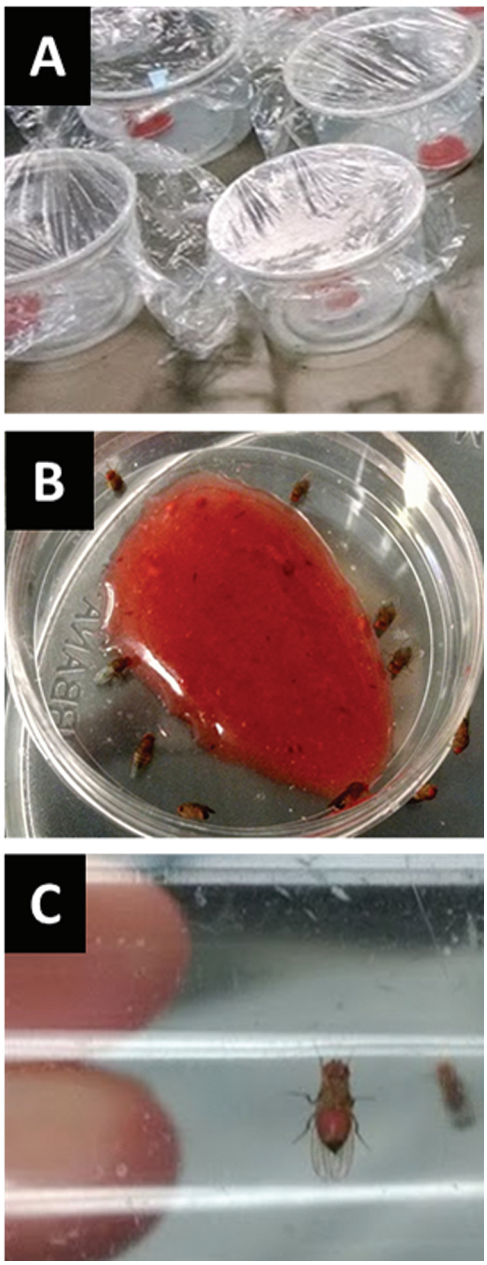

Fig. 1. *Drosophila suzukii* feeding assay: (A) feeding arenas, (B) flies feeding on strawberry puree, and (C) fly with pink abdomen, visual confirmation of fruit ingestion.

DNA Extraction

Flies were removed from 70% ethanol, surface-washed in a 5% bleach solution, and rinsed twice in distilled water (modified from Cooper et al. 2016). Flies were then decapitated to minimize interference during the DNA amplification from DNases found in their heads (J. Chiu, personal communication). DNA was then extracted from the remaining bodies of 10 of individual flies per time point using DNeasy blood and tissue kit (catalog no. 69506, Qiagen Inc., Germantown, MD, www.qiagen.com).

Primer Selection

We utilized a strawberry-specific primer (fwd: GGATTC TTCCTGGTTGAGACC; rev: CCGGCTACCTTATGGTTCTTC) (Dong et al. 2015) to amplify a 169-bp region of plastid DNA. Prior to our experiments, we tested target specificity against DNA extracted from other crop hosts including raspberry, blackberry, and blueberry. Primers available for purchase for these hosts were also explored, but none yielded reliable DNA amplification. *D. suzukii*-specific primers (fwd: AATTGTTACCGCACATGC; rev: GGAATGCTATATCTGGGTCC) were selected, which amplify a 117-bp region of the COI gene (Dhami and Kumarasinghe 2014). This provided detection of *D. suzukii* DNA in all samples tested, ensuring that DNA had been extracted from individuals.

DNA Amplification and Comparative C_T

Quantitative polymerase chain reaction (qPCR) reactions were performed in triplicate in 96-well plates (USA Scientific catalog no. 1402-9100) with strawberry primers or *D. suzukii* primers. Each well contained 1 µl of extracted DNA, 0.3 µl of forward primer, 0.3 µl of reverse primer, 8.4 µl dH_20, and 10 µl of iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, CA) for a total volume of 20 µl per well. Amplification was performed using an Applied Biosystems QuantStudio 6 Flex System in Fast mode preset for SYBR reagents in a comparative C_T experiment. Amplified products were then subjected to melt curve analysis to confirm identity. Each plate contained the following controls: 1) DNA extracted from starved flies, 2) strawberry DNA, and 3) no DNA water control. Amplification and melt curve analysis were performed for a minimum of three flies per time period.

Results

Food Resource Detection

Melt curves were created for the products amplified during qPCR. Products amplified by the *D. suzukii*-specific primers melt at approximately 76°C (Fig. 2A); these were used as a control to ensure that *D. suzukii* DNA was extracted from the fly. The amplified region of strawberry DNA melts at approximately 72.5°C (Fig. 2B), and

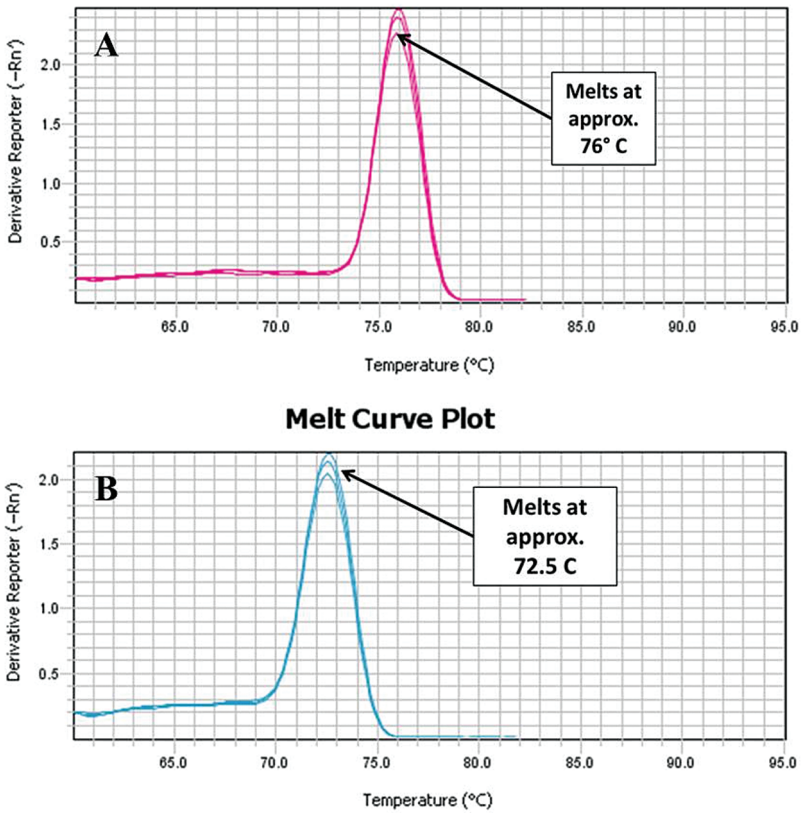

Fig. 2. Melt curve plots for (A) *Drosophila suzukii* and (B) strawberry DNA from quantitative polymerase chain reaction. Specific temperature peaks (A, B) enable identification of amplified product.

the presence of this peak shows that the target region of strawberry DNA was amplified. Lack of this peak means that the target region was not amplified.

Duration of Detection

Ingested strawberry DNA was detectable at all time points tested (Table 1, Fig. 3). This suggests that DNA from this host food is detectable for at least 1 wk post-consumption under laboratory conditions (~23°C, 16:8 [L:D] h) even in the presence of additional food resources.

Discussion

Our data show that gut content analysis may be a viable option for assessing direct feeding resource association for *D. suzukii* based on laboratory tests. Further exploration is necessary to 1) develop DNA primers specific to other highly impacted berry crops and 2) determine appropriate trapping techniques, including duration of trapping, for use of this tool in a field setting. *D. suzukii* DNA has been extracted from flies trapped and preserved in the field in propylene glycol for up to 7 d (Abrieux and Chiu, personal communication); therefore, it is likely that fruit DNA could also be detected in such flies.

In this study, we focused on detailing the duration of strawberry DNA detection post-consumption as we were able to use a published DNA primer that was available for purchase that did not amplify DNA of any of the other fruit hosts tested (blueberry, blackberry, and raspberry). Available primers for the other hosts were not as reliable, either not amplifying DNA of the target host or amplifying DNA from several hosts. To expand this study into other cropping systems, we will need to develop primers that are specific to these additional fruit, which is beyond the scope of the current project.

Table 1. Number of *Drosophila suzukii* confirmed to contain strawberry DNA at specific time points after ingestion and the threshold cycle (C_T) for detection of this DNA

Time point (h)	Number of *D. suzukii* tested	Number of *D. suzukii* confirmed for strawberry DNA
Unfed control	3	0
0	3	3
2	3	3
4	4	3
6	4	4
8	4	3
10	3	2
12	3	3
24	3	3
48	5	4
120	3	2
168	5	4

Presence of both the target strawberry and *D. suzukii* amplification products were detected at all time points tested (Table 1; Fig. 3, subset of time points). We chose to focus on detection of a crop host because we have observed likely host feeding under field conditions. Detecting and determining the proportion of flies that fed upon host crops both within and outside of these plantings, and distance fed flies are trapped from plantings, can inform our understanding of *D. suzukii* local resource use and movement in relation to a focal crop, and inform management decisions. For example, if flies remain within a crop field while resources are

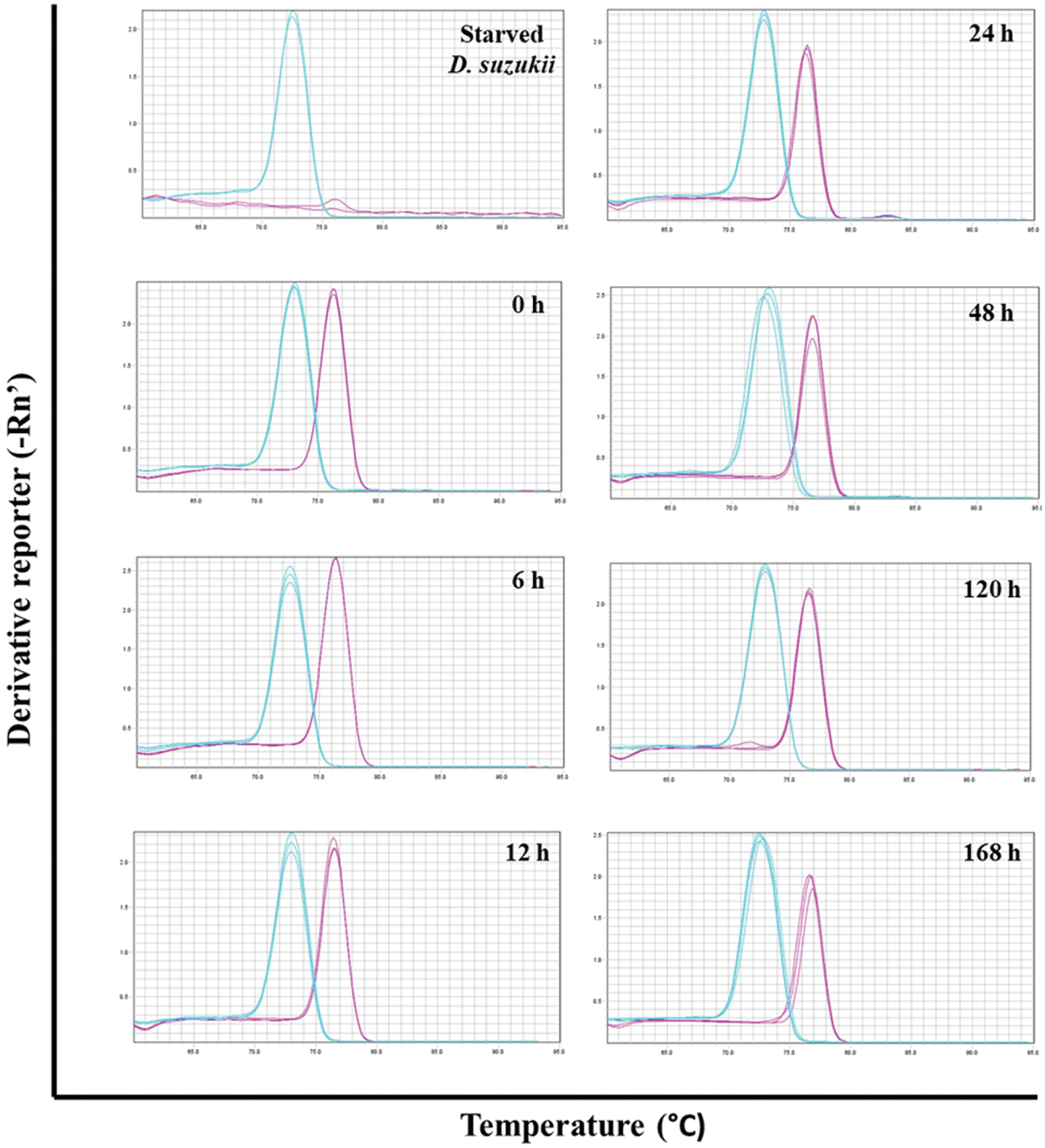

Fig. 3. Melt curve plots for the target product amplified from strawberry DNA (pink) from quantitative polymerase chain reaction for a subset of time points after feeding (starved, 0, 6, 12, 24, 48, 120, and 168 h). Melt curves for the target product from *Drosophila suzukii* (blue) amplification are overlaid with those from strawberry. The presence of *D. suzukii* DNA in each sample confirms that DNA was extracted from these flies.

available versus moving in and out frequently, field management regimes can be modified to include incorporation of cultural management to reduce the availability of ideal microhabitats or refuges within a crop that enable flies to persist despite management actions (Diepenbrock and Burrack 2016).

Deciphering key feeding associations for adult *D. suzukii* will enable a better understanding of their local resource use and help inform management of this pest by altering the ability of adult flies to access these resources (e.g., removal and targeted trapping techniques). *D. suzukii* remain present and can be trapped in the southeast United States throughout the winter when no crop resources are available (Diepenbrock, unpublished data), and it is unclear what nutritional resources are necessary for survival during this period. Eventually, gut content analysis could be used to determine which noncrop resources adult flies are using by employing DNA barcoding (sensu Jurado-Rivera et al. 2009). Initial efforts, however, are focused on their use of a specific focal crop (strawberry in this study), to decipher how *D. suzukii* use that crop during the production period.

In contrast with other methods to track the movement of insects, such as mark-recapture studies, analyzing gut content confirms a direct feeding relationship of that insect with a specific host. The ability to describe the direct resource associations of mobile polyphagous plant-feeding insects such as *D. suzukii* opens up new avenues into studying the ecology of pest insects. And in the example of an invasive pest, can expedite the process of understanding its ecology

and assist in the development of ecologically suitable management tactics.

Acknowledgments

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